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14. ABSTRACT

The goal of the GWI consortium is to develop a better understanding of GWI and identify specific disease targets to find treatments that will address the cause of the disease. The consortium will integrate our clinical understanding of the disease process with basic research efforts using a novel mathematical model. The computational biology approach will enable the consortium to quickly identify targets of dysfunction and find treatments that will address the causes of the disease. The project will combine animal models of GWI with focus on the immune, cardiovascular and autonomic systems.

15. SUBJECT TERMS

Nothing listed

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INTRODUCTION

The underlying mechanisms of GWI remain unknown and treatment has been palliative, symptom-driven and physician-directed. The purpose of this multidisciplinary consortium project is to investigate animal GWI models with the goal of testing chemical treatments. The immune and autonomic biomarkers will be tested using a computational modeling approach allowing for a critical analysis and an accurate selection of test agents. The idea is to combine animal and human studies – a translational approach. Animal studies will be followed by clinical trials with agents thought to be most efficacious.

KEYWORDS

Autonomic Dysfunction
Computational Biology
Cytokines
Deregulated Balance
Diisopropyl Phosphorofluoridate
Electrocardiogram
Gulf War Illness
Homeostasis
Molecular Targets
Mouse Model
Putative Therapeutics
Regulatory Network Configuration
Repurposed Drugs
Sarin
Stress Response
Target Intervention
Therapeutic Interventions
Translational Human Clinical Trials
Translational Model
Treatment

ACCOMPLISHMENTS

What were the major goals of the project?

	Timeline (Months)	Percentage Complete
Major Task 1: Setup the administrative structure required for the conduct of the animal and human studies		
Subtask 1: Prepare Regulatory Documents and Research Protocols for Study 1		
Prepare, submit and receive approval for animal protocols	1-4	100%
Refine experimental protocols via conduct of preliminary experiments.	4-12	100%
Refine eligibility criteria, exclusion criteria, screening protocol	3-12	100%
Finalize consent form & human subjects protocol	3-12	100%
Submit amendments, adverse events and protocol deviations as needed	As Needed	100%
Coordinate with Sites for annual IRB** report for continuing review	Annually	0%
Subtask 2: Establishment of administrative structure including coordinating center and database system		
Recruit, hire and train key personnel, students, staff and faculty	1-6	100%
Setup the coordinating center including database setup	1-12	100%
Setup administrative including committee appointments and scheduling of key review meeting	1-5	100%
Development of reporting procedures – minimum of updates every 6 months.	3-12	100%
Finalize consent form & human subjects protocol , receive approval	24-36	50%
Annual meeting with the consortium members and the external advisory committee – live and via internet	As scheduled	100%
Meetings in the DC region with DoD staff and representatives of the groups – twice per year	As scheduled	100%

	Timeline (Months)	Percentage Complete
Major Task 2: Refinement and enhancement of animal models for GWI.		
Sub task 1: Establish the model of autonomic dysfunction as a surrogate for GWI.		
Train staff and students in specialized surgical methods used to setup for monitoring autonomic function.	Begin 3 and continue	100%
Test cholinergic toxins in mice with examination of peripheral autonomic and cardiac function – predict long term deficits	4-15	40%
Employ spectral analytical methods for examination of sympathetic and parasympathetic balance	Begin 4 continue	100%
Conduct wheel running acute and chronic exercise tests to simulate the exercise model in humans	4-12	100%
Combine tests of acute and chronic exercise in the GWI chemical toxin model, providing an excellent preclinical comparison.	12 continue	50%
Submit animal protocol amendments as required	As needed	100%
Measure immune biomarkers in the autonomic dysfunction model, compare to measures of adrenal function	24 continue	15%
Extend preliminary analysis to transcriptional level. Filter and normalize data using accepted best practices and perform traditional analysis of expression profiles at the level of individual genes	6-18 continue	30%
Successful use of data coordination/statistical analysis center bringing together large amounts of data from multiple systems	5 continue	20%
Subtask 2: Establish the model of DFP/CORT as a surrogate for GWI.		
Train staff and students in conduct of model	Begin 3 and continue	100%
Test cholinergic toxins in mice with examination of immune markers in brain and periphery	4-15	50%
Employ analytical methods for examination of immunological balance	Begin 4 -16	60%
Establish the minimum levels of corticosterone required to maintained a heightened pro-inflammatory response to the sarin surrogate, DFP	4-12	100%
Evaluate stress regimens to establish protocols required to exacerbate proinflammatory response to sarin surrogate, DFP	5-15	70%
Submit animal protocol amendments as required	As needed	100%

	Timeline (Months)	Percentage Complete
Subtask 3: Characterize the molecular and cellular phenotypes of GWI mouse models with the idea of using them to test treatments.		
Use transcriptional analysis to study the immunological basis for the brain and blood changes in the GWI models	12 -24	50%
Use bioinformatic method to estimate pathway activation from gene expression and conduct comparisons between mouse and humans.	6-18	50%
Use molecular modeling to identify and develop networks of expression allowing for robust comparisons between GWI and animal models. Test under baseline and stimulated (stress hormones or exercise)	12-30	70%
Major Task 3: Identification of Illness specific networks with focus on human and mouse comparisons		
Subtask 1: Conduct network analysis for humans and animal models		
Apply biological modeling techniques to pathway activation computed in task 2 sub 3 to render pathway networks	6-12	70%
Integrate with other levels of biology then identify and compare functional modules at various resolutions across groups	12 -18	70%
Conduct detailed analysis of network topology applying measures of network structure and information flow to identify critical information-processing modules	12-24	70%
Conduct an analysis of the alternate steady states available to the regulatory networks identified in human and mouse models.	12-30	60%
Inform pathway-specific genomic panel based on the key network regulatory pathways	12-30	85%

	Timeline (Months)	Percentage Complete
Major Task4: Large-scale simulation of treatment.		
Subtask 1: Conduct in silico sensitivity analysis and rank candidate target nodes		
Use simulation experiments to assess and rank the impact of introducing an in silico equivalent standardized treatment pulse or pulse train at each node in turn throughout the model network	18-30	60%
Rank the candidate target nodes in terms of their relative contribution to shifting the structure of the network recovered under treatment and the network presented in healthy control subjects	18-30	60%
Major Task 5: Define and deploy large-scale optimization.		
Subtask 1: Evaluate and select the best global search algorithm for targeting intervention possibilities		
Review latest developments in evolutionary programming techniques as well as hybrid gradient-based techniques to determine the most suitable search algorithm. Acquire or develop code and deploy.	12-18	50%
Configure simulation-based optimization scheme that evaluates the fitness of candidate interventions by repeatedly launching short network simulation runs in search of the most robust treatment course	18-24	60%
Major Task 6: Identify candidate treatment courses for GWI		
Subtask 1: Using task 5 launch optimization runs from multiple initial conditions of endocrine-immune status		
Identify and describe mathematically the immune and endocrine descriptors that can be effectively and safely changed and over what range they may be changed.	24-30	45%
Using drug databases and bioinformatic techniques identify drugs currently available for repurposing to treat GWI	12-30	50%
Search for novel treatment courses. Launch repeated searches for optimal treatments using the set of candidate cytokine, hormone/autonomic and immune markers isolated in task 5	24-36	15%

	Timeline (Months)	Percentage Complete
Major Task 7: Identify candidate treatment courses for GWI		
Subtask 1: Select and test pharmacological therapies on basis of data from computational models in animals		
Use previous data to select best animal models based on immunological and autonomic biomarkers	24-36	60%
Develop computer/mathematical paradigms for evaluation of treatment strategies	12-30	50%
Develop pilot clinical trials on basis of animal studies	24-36	40%
Major Task8: Verify treatment effectiveness in human subjects		
Subtask 1: Studies of treatment effectiveness in humans		
Design assessment platform for use in human translational studies using the RedCAP platform as a foundation	18-24	100%
Complete the IRB process for selected study drugs, using the Miami VAMC IRB with OCMR review.	24-30	50%
Recruit and perform assessments of GWI subjects on intervention(s) in the phase 1 translational studies.	30-40	0%
Evaluate change in network interactions from interventions suggested Study 3 and 4. Inform the model with the human study data and refine as necessary	32-48	0%

What was accomplished under these goals?

- Broderick group recruited Hooman Sedghamiz (M.Sc. 2014, University of Linkoping, Sweden), effective July 11, into position of data modeler supported under the concurrent sister project GW140142. **(Task 1; Subtask2)**
- Dr. Amanpreet Cheema joined the GWI consortium as program administrator. **(Task 1; Subtask2)**
- Three post-doctoral members in Dr. James O'Callaghan's Lab presented posters on data gathered from relevant consortium related projects at the Society for Neuroscience conference in Chicago, IL (12-10-2015 to 15-10-2015), and at the Society of Toxicology conference in New Orleans, LA. (13-3-2016 to 17-3-2016) **(Task 1; Subtask2)**
- Drs. Broderick and Craddock presented detailed review of numerical protocol to Dr. Reifman and other members of EAB at annual review in Fort Detrick on Oct. 26-27, 2015. **(Task 1; Subtask2)** Highlights include:
  a) Confirmation of feasibility of the proposed numerical protocols involving immune signaling network analysis. Analysis pointed to significant differences in immune signal propagation occur as a result of DFP exposure potentiated by corticosterone.
  b) Results of a first preliminary comparative analysis of cytokine expression profiles in

blood from human GWI veterans with profiles measured in blood of exposed and LPS-challenged mice at 21 days (the basic mouse GWI model).

- Dr. Broderick visited CDC/ NIOSH laboratories of Drs. O'Callaghan and Miller to Nov. 08-12, 2015, during which:
  a) Conducted a review of latest PCR gene expression and NanoString data from Dr. Nathanson, rationalizing a cohesive plan for continued analysis that may be articulated as a structured manuscript. **(Task 3; Subtask1)**
  b) Conducted a review of the latest simulation data from the neuro-inflammation model and resulting manuscript prepared with Dr. Craddock and other members of the CSB. **(Task 4; Subtask1)**
  c) Brainstormed with the Miller and O'Callaghan teams to design animal trials of candidate treatment protocols developed using computer simulations **(Task 7; Subtask1)**. These include a 2-step application of Daclizumab or Enbrel followed by Mifepristone or Minocyclin prior to LPS challenge at 21 days post-DFP/Cort exposure.
- Hosted on-site visit of SWRI team members for 2-day brainstorming session to review current candidate drug targets and develop methodological approach to computer-based drug screening (Sep. 24-25, 2015). **(Task 1; Subtask2)**
  a) SWRI team members under the leadership of Dr. Jon Bohman; used proprietary Rhodium platform to perform screening of Pubchem listed compounds and FDA-MDD approved drugs in molecular docking simulations against TNFa and glucocorticoid receptor (GCR) targets. Results point to Amlexanox for GCR; Orlistat for TNFa.
  b) INIM/ CSB members under the direction of Dr. Craddock; concurrently used a consensus docking scheme based on Autodock4, Vina, and Glide with DrugBank indexed FDA-approved drugs against GCR, estrogen receptor, androgen receptor as well TNFa and IL2 both the molecule and the receptors.
- Data Manager post was advertised to replace Mr. Rice following his migration (Oct. 2015) to the concurrent sister project GW140142 (Broderick, PI) also known as the "fight or flight" expansion award. Currently a candidate has been selected. **(Task 1; Subtask2)**
- Morris group's research associate Jacqueline Machi received a travel award from the European Society of Cardiology to attend the August meeting in Rome. She will present her studies on the cardiac effects of DFP:
  "A novel model for autonomic dysfunction using chronic exercise and organophosphate treatment". Machi JF, Salgueiro L, Conti F, Morris M. Institute of Neuro-Immune Medicine, Nova Southeastern University, Fort Lauderdale, Florida. ESC 2016. **(Task 1; Subtask2)**
- Jacqueline and Rodrigo from Morris group presented a poster and gave an oral presentation, respectively, at the Eastern-Atlantic Student Research Forum -University of Miami Miller School of Medicine 03-02-2016 to 03-05-2016. Published an abstract (DFP interactions with exercise stress) at the Experimental Biology meeting (EB) 4/2016 in San Diego:
  "Low-Intensity Exercise Training Improves Autonomic Modulation and Cardiac Function in a Potential Model of Gulf War Illness in mice with Organophosphate (DFP) Exposure: Analyze by Noninvasive Method of Cardiovascular Measures". Machi JF,

Conti F, Hernandez D, Salgueiro L, Klimas N, Fletcher MA, Morris M. Nova Southeastern Univ. and Miami VA Healthcare Syst. EB 2016. **(Task 1; Subtask2)**

- Dr. Morris attended to 55th Annual Meeting in Toxicology (Society of Toxicology), March 13–17, 2016, in New Orleans, Louisiana. **(Task 1; Subtask2)**
- A daylong conference was presented by Drs. Nancy Klimas (NSU), Gordon Broderick (NSU), Jeff Cournoyer (NSU), and Kimberly Sullivan (Boston) on Gulf War Illness Conference: A 25 Year Journey of Hope and Progress; the conference assessments reflected feedback that let us know we achieved that goal. Morris GWIC and Sullivan GWIC participated in this conference organized by Dr. Klimas and her VA team on February 29, 2016. **(Task 1; Subtask2)**
- The Miami VAHCS Institutional Animal Care and Use Committee (IACUC), and the local Biosafety committee had approved the amendments for the use of Lipopolysaccharides of E. coli (LPS) to stimulate a strong neuroimmune response in mice pre-exposed and primed with organophosphates (Sarin or the surrogate DFP exposure) and the use of therapeutic agents (Enbrel and Mifepristone). **(Task 2; Subtask2)**
- Members of the Consortium submitted and have received acceptance of 2 poster presentations at the upcoming International conference on Traumatic Stress Research: Enabling Bedside Implementation, jointly sponsored by the International Society for Traumatic Stress Studies (ISTSS)/ Canadian Institute for Military Veteran's Health Research (CIMVHR) in Toronto, Canada, May 9-10, 2016:

  a) Vashishtha S, Russell L, Michalovicz L, Kelley KA, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M, Broderick G. Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model. Int Soc for Traumatic Stress Studies (ISTSS)/ Canadian Inst for Military Veteran's Health Research (CIMVHR) Meeting, Traumatic Stress Research: Enabling Bedside Implementation, Toronto, Canada, May 9-10, 2016; Poster session.

  b) Toole JT, Rice MA jr., Nierenberg B, Craddock TJA, Fletcher MA, Klimas NG, Zysman J, Morris M, Broderick G. Increasing Resilience to Traumatic Events: Simulating the Protective Role of Well-Being. Int Soc for Traumatic Stress Studies (ISTSS)/ Canadian Inst for Military Veteran's Health Research (CIMVHR) Meeting, Traumatic Stress Research: Enabling Bedside Implementation, Toronto, Canada, May 9-10, 2016; Poster session. **(Task 1; Subtask2)**

  - Jacqueline Machi from Morris group received
    a) Young Investigator Basic Science Presentation on Exercise Training Improves Cardiac Function in Mice Exposed to a Model of Gulf War Illness: Combined use of Corticosterone and Diisopropylfluorophosphate (DFP). Miami VA Research day poster session. May 10, 2016.
    b) ESC Congress Educational Grants. This grant aims to encourage and support the development of the professional careers towards the fight against cardiovascular disease. ESC: Italy Rome 2016. **(Task 1; Subtask2)**
- Broderick group published a manuscript as part of concurrent efforts funded under affiliated project GW093042 (Broderick, PI) and its ongoing expansion award GW140142 (Broderick, PI) in the journal Systems Biomedicine (Taylor and Francis): Rice M, del Rosario R, Craddock TJA, Barnes ZM, Klimas NG, Fletcher MA, Zysman J,

Broderick G. Gulf War Illness: Is There Lasting Damage to Endocrine-immune Circuitry? Sys Biomed 2016, 2(4): 80-89. **(Task 1; Subtask2)**

- INIM team submitted abstracts for oral and poster presentations at The Gulf War Illness Symposium jointly held with IACFS Annual Meeting in October, 2016 in Fort Lauderdale, FL. **(Task 1; Subtask2)**
  a) Broderick G, Vashishtha S, Russell L, Michalovicz L, Kelley KA, Vrana JA, Locker AR, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M. Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model of Gulf War Illness.
  b) Toole JT, Rice, MA Jr., Cargill J, Craddock TJA, Nierenberg B, Klimas NG, Fletcher MA, Morris M, Zysman J, Broderick G. Increasing Resilience to Traumatic Stress: Understanding the Protective Role of Well-Being.
  c) Craddock TJA, Harvey JM, Nathanson L, Barnes ZM, Klimas NG, Fletcher MA, Broderick G. Using gene expression signatures to identify novel treatment strategies in gulf war illness.
  d) Jaundoo R, Bohmann J, Gutierez G, McDonough J, Klimas NG, Broderick G, Morris M, Craddock TJA. Structure-Based Repurposing of FDA-Approved Drugs to Identify Specific Small Molecule Inhibitors of TNF-alpha, IL-2, and the Glucocorticoid Receptor for Treatment of Gulf War Illness.
  e) Cournoyer J, Broderick G, Collado F, Fletcher MA, Klimas NG. The Use of the Respiratory Exchange Ratio in Assessing the Metabolic Efficiency of Patients with ME/CFS and GWI.
- Broderick group presented 2 posters at the International conference on Traumatic Stress Research **(Task 1; Subtask2)**: Enabling Bedside Implementation, jointly sponsored by the International Society for Traumatic Stress Studies (ISTSS)/ Canadian Institute for Military Veteran's Health Research (CIMVHR) in Toronto, Canada, May 9-10, 2016:
  a) Vashishtha S, Russell L, Michalovicz L, Kelley KA, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M, Broderick G. Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model. Int Soc for Traumatic Stress Studies (ISTSS)/ Canadian Inst for Military Veteran's Health Research (CIMVHR) Meeting, Traumatic Stress Research: Enabling Bedside Implementation, Toronto, Canada, May 9-10, 2016; Poster session.
  b) Toole JT, Rice MA jr., Nierenberg B, Craddock TJA, Fletcher MA, Klimas NG, Zysman J, Morris M, Broderick G. Increasing Resilience to Traumatic Events: Simulating the Protective Role of Well-Being. Int Soc for Traumatic Stress Studies (ISTSS)/ Canadian Inst for Military Veteran's Health Research (CIMVHR) Meeting, Traumatic Stress Research: Enabling Bedside Implementation, Toronto, Canada, May 9-10, 2016; Poster session.
- On April 8th, 2016, Dr. Craddock gave a presentation at the Advancing Computational Biology Symposium at Howard University in Washington, DC. The topic was 'Using genomic drug repurposing and docking to predict novel treatment avenues for Gulf War Illness'. **(Task 1; Subtask2)**
- On Dec 11, 2015, CDC presented 4 posters on relevant consortium related data at Society for Neuroscience conference in San Diego, CA. **(Task 1; Subtask2)**

- Members of the Consortium hosted GWI Chronic Fatigue symposium cosponsored by the two GWIC (Sullivan and Morris) at the IACFS/ME conference in October: Dr. Lidie Chaired the symposia. **(Task 1; Subtask2)**
- Broderick group currently is preparing manuscript titled 'Structure-Based Repurposing of FDA-Approved Drugs to Identify Specific Small Molecule Inhibitors of TNF-alpha, IL-2, and the Glucocorticoid Receptor for Treatment of Gulf War Illness'. Rajeev Jaundoo, Jonathan Bohmann, Gloria Gutierez, Joe McDonough, Nancy G. Klimas, Gordon Broderick, Mariana Morris and Travis J.A. Craddock
- Members of GWIC have submitted 9 articles which are under review. **(Task 1; Subtask2)**
  a) Genomic approach to find mechanisms of Gulf War Illness pathobiology. Nathanson L., Rashid H., Collado F., Cash M., Saladin B.F., Perez A., Fletcher M.A., Klimas N.G.
  b) Cardiac Function in a murine Model of Gulf War Illness (GWI): Combination of Organophosphate (DFP) and Exercise Training Jacqueline F. Machi, Luis M. Salgueiro, Filipe F. Conti, Rodrigo Schmidt, Mariana Morris.
  c) Murine echocardiography offers an effective method for noninvasive evaluation of autonomic balance as measured by heart rate variability Rodrigo Schmidt, Jacqueline F. Machi, Filipe F. Conti, Luis M. Salgueiro, Mariana Morris.
  d) Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model of Gulf War Illness. Broderick G, Vashishtha S, Russell L, Michalovicz L, Kelley KA, Vrana JA, Locker AR, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M
  e) Increasing Resilience to Traumatic Stress: Understanding the Protective Role of Well-Being. Jonathan T. Toole, Mark A. Rice, Jr., Jordan Cargill, Travis J. A. Craddock, Barry Nierenberg, Nancy G. Klimas, Mary Ann Fletcher, Mariana Morris, Joel Zysman, Gordon Broderick
  f) Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model of Gulf War Illness. Broderick G, Vashishtha S, Russell L, Michalovicz L, Kelley KA, Vrana JA, Locker AR, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M
  g) The Use of the Respiratory Exchange Ratio in Assessing the Metabolic Efficiency of Patients with ME/CFS and GWI. Jeffry Cournoyer
  h) Persistently elevated bone marrow somatic mutation as a biomarker of clinically relevant exposures in Gulf War Illness Grant SG, Latimer JL, Sveiven S, Fletcher MA, and Klimas NG.
  i) Understanding Gulf War Illness: Brain-Immune Biomarkers, Cognitive Functioning and Treatment Development Strategies 25 Years after the War. Sullivan K, Abou Donia M, Krengel M, Klimas N, Golier J, Meggs W and White RF.
- The Gulf War Illness Research Program (GWIRP) featured CDC progress, especially the development of animal models under GWIRP award, GW080150 and GW060050 on the CDMRP website (http://cdmrp.army.mil/gwirp/default.shtml). **(Task 1; Subtask2)**
  a) O'Callaghan JP, Kelly KA, Locker AR, Miller DB, Lasley SM. 2015. Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of

Gulf War Illness. J Neurochem 133(5):708-721.

b) Oswal DP, Garrett TL, Morris M, et al. 2013. Low-dose sarin exposure produces long term changes in brain neurochemistry of mice. Neurochem Res 38(1):108-116.

- The consortium hosted preconference on our team's progress in GWI and ME/CFS at NSU: we had 1000 attendees between the webcast and onsite registration! On site included the policy making program officers at the DoD, NIH, VA and private foundations and 150 scientists, clinicians and advocates. Around 858 logged in to our weblink available for viewing for no charge. GWI veterans approached the GWI consortium and provided valuable input. 3 national stories underway by reporters who attended the conference. DoD program officer's (Dr. Lidie) comments: "The meeting was excellent and the science stellar. Thank you very much, Nancy, for inviting me to be part of it and to the entire INIM team for being such great hosts!"
- Six LOI's involving this group, all a direct result of the consortia's team building efforts went out on July 15 in response to the GWI CDMRP call for proposals and final proposals were submitted in Oct 2016 **(Task 1; Subtask2)**
- CX3CR1 -/- mice were bred at the CDC for pilot microglial response study **(Task 2; Subtask1)**
- Pilot DFP study was completed to assess humane endpoints and palliative care options in regards to ACUC/AV concerns over DFP mortality at the CDC. 9-12-2015 **(Task 2; Subtask2)**
- The methods for use of DFP in mice has been set up. This included approval of methods for use of DFP, chemical toxin, by the VA committee, Chemical Hygiene and Biosafety. Important issues are related to DFP purchase, storage, dilution, exposure (injection). **(Task 2; Subtask1)**
- Male mice were tested for LD50 of SC DFP exposure providing information on the appropriate dose to use in the modeling studies. The physiological effects of DFP in sedentary and exercise stressed mice were tested. The focus was on the influence of DFP on cardiac (Echo), autonomic (spectral analysis), immunological and body composition parameters. Results suggest cardiac changes associated with DFP exposure. **(Task 2; Subtask2)**
- CDC exposed CX3CR1 -/- mice to CORT LPS and performed a short-term GWI exposure paradigm (3-5 weeks) (1-1-2016 - present). **(Task 2; Subtask2)**
- Dosing pilots performed by CDC for etanercept and mifepristone, FDA-approved drugs identified to be useful in combination for the re-set of the GWI phenotype by systems biology analysis (8-3-2016 to present) along with starting the GWI treatment studies using the combinations of etanercept and mifepristone, and minocycline and mifepristone (11-8-2016 to present). **(Task 2; Subtask2)**
- CDC has been accepted to present on GWI as part of a symposium on sickness behavior-related illnesses at the Society of Toxicology conference in Baltimore, MD. (12-3-2017 to 16-3-2017). **(Task 1; Subtask2)**
- INIM received the modular phenotyping platform for the synchronized assessment of metabolic, behavioral, and physiological parameters, including O2/CO2 and telemetry and will be set at NSU at the end of the year. **(Task 1; Subtask2)**
- The evaluation of a combined therapy with Etanercept and Mifepristone on the decline of cardiovascular function of mice exposed to the experimental model of GWI (CORT+DFP) is in progress. **(Task 7; Subtask1)**

- The development of protocols for use of chemical treatments is in progress. The amendment for the inclusion of Enbrel and Mifepristone passed the first review 04-01-2016. Kristina Aenlle (IACUC director) expedited the amendment. SARIN protocol is under review by ICD. They requested details on mice transportation. **(Task 7; Subtask1)**
- Echocardiography (ECHO) and electrocardiogram (ECG) with dobutamine stress test were performed before DFP injection (5 months of exercise training or resting) and after DFP injection (6 months of exercise training or resting) and increased plasma levels of KC and IL-6 were observed after DFP exposure at the sedentary group (see Fig 1. Appendices). **(Task 2; Subtask 1)**
- CDC performed a comparative study of 3 and 5 weeks long GWI "phenotype" exposure paradigms, where mice received 7 days of CORT (every other week) for the specified time. In addition to providing more points for intervention (i.e. 3 intervening weeks between CORT DFP, and CORT LPS exposures), the 5-week study demonstrated a more consistent trend of greater inflammatory expression in groups exposed to CORT DFP LPS over CORT LPS, compared to the 3-week cohort. Thus, the 5-week paradigm will be used for screening potential treatments. (See appendices Fig 2.). **(Task 2; Subtask 2)**
- CDC performed short term treatment study was conducted to examine efficacy of minocycline administration post-DFP dosing but prior to an inflammagen challenge. Treatment with minocycline 1 hour prior to LPS exposure (after initial CORT and DFP exposure) significantly reduced neuroinflammation in almost all markers in our panel (See appendices Fig 3.). **(Task 2; Subtask 2)**
- CDC performed a short-term, one-week study of GWI exposures in CX3CR1 -/- mice. In this experiment, mice were exposed to corticosterone in the drinking water (CORT; 200 mg/L in 0.6% EtOH) for 7 days, followed by a single injection of DFP (4 mg/kg, i.p.). Two days following DFP exposure, mice were challenged with LPS (0.5 mg/kg, s.c.) and sacrificed 6 hours later. From this study, we found that dampening of the reactivity of microglial by the knockout of CX3CR1 prevented the enhance neuroinflammation induced by CORT DFP LPS exposure (compared to C57BL/6J mice; see Fig. 3 Appendices) (see Fig 4 Appendices). This is the first direct evidence that has been collected that support a role for microglial activity in the GWI phenotype. **(Task 2; Subtask 2)**
- CDC performed a treatment study using the 5 week GWI paradigm in C57BL/6J mice. In this experiment, mice were exposed to corticosterone in the drinking water (CORT; 200 mg/L in 0.6% EtOH) for 7 days, followed by a single injection of DFP (4 mg/kg, i.p.). Mice were given corticosterone every other week for a total of 5 weeks followed by an LPS challenge (0.5 mg/kg, s.c.) and sacrificed 6 hours later (see paradigm in Fig 5). Treatments were provided during the 3rd week (during CORT exposure) or the 4th week (between CORT exposures). The treatments were as follows: Enbrel (5 mg/kg, s.c.) + Mifepristone (20 mg/kg, i.p.), and Minocycline (100 mg/kg, s.c.) + Mifepristone (20 mg/kg, i.p.). Enbrel and Mifepristone (EM) were given two days apart, and Minocycline and Mifepristone (MM) were given one day apart. The results of this study indicated that the Enbrel/Mifepristone combo treatment, given during CORT exposure significantly reduced the level of neuroinflammation instigated by the GWI paradigm (see Fig 5 Appendices). **(Task 2; Subtask 2)**

- Morris group observed significant reduction in cardiac output (CO), end diastolic area (EDA), end systolic area (ESA), and increased E/A ration (Fig 6 appendices) in mice exposed to corticosterone (CORT) and the organophosphate, diisopropyl fluorophosphate (DFP). Data from echocardiography (ECHO) was used to evaluate autonomic function using heart rate variability method. This provided information on parasympathetic (HF) and sympathetic (LF) modulation. An increased sympathetic modulation as well as a decreased vagal modulation, evidenced by LF and HF respectively, was observed after DFP exposure on the autonomic system (Fig 7). Morris group found significant increase in LF/HF ratio of the sedentary group after DFP exposure when compared with LH/LF ratio before DFP exposure (Fig 7). No significant increase in LF/HF ratio after DFP exposure for the trained group was seen. This comportment is due to a significant increase in sympathetic modulation (LF) after DFP exposure on the sedentary group, which does not happen in trained group. Further, no significant decrease on parasympathetic modulation (HF) for bough groups was observed between before and after DPF exposure. **(Task 2; Subtask 1)**
- Morris group performed immunological measurements using ELISA kits. Data is presented for Keratinocyte Chemoattractant (KC) chemokine and Interleukin –6 (IL-6). Increased plasma levels of KC and IL-6 were observed after DFP exposure, as shown in Fig 8. The synthesis of the neutrophil-recruiting chemokines is an important step in the initiation of an immune response. **(Task 2; Subtask 1)**
- Morris group conducted a long term (5 months) treatment (exercise) study to examine efficacy of pre training in DFP exposure male. Male C57BL/6 mice were divided in two groups (n=8) as follows: Trained (T): Receiving exercise training on an electronic wheel. Sessions were one 1 hr. day, 5 days/week. Sedentary (S): This group did not receive exercise training sessions. CORT+DFP exposure were performed after 6 months of exercise training. Briefly, CORT (200 mg/Lt in drinking water) was provided during 7 days. On day 7, a single dose of DFP (1.5 mg/kg) was administrated subcutaneously. We are presenting cardiovascular evolution before and after DFP exposure. Results from this study revealed that exercise preserve some cardiac functions after DFP how we can see in details in the figure below (Figure 9). The sedentary group after exposure significantly reduced cardiac output (CO), end diastolic area (EDA), end systolic area (ESA), Left ventricle mass (LVM) and increased E/A ration, ejection fraction (EF) and fraction shortening (FS). Thus, DFP plus exercise training could provide an excellent model for study long term consequences of autonomic disease as seen in GWI. **(Task 2; Subtask1)**
- Morris group observed increased plasma levels of KC and IL-6 were observed after DFP exposure at the sedentary group, as shown in Fig 10. The synthesis of the neutrophil-recruiting chemokines is an important step in the initiation of an immune response. Finally, we can observe that trained group did not have alteration on KC and IL6. **(Task 2; Subtask 1)**
- Morris group conducted dobutamine stress test after DFP exposure which showed that the sedentary group needed an adaptation from the heart to keep the normal functional for this stress (dobutamine), increasing isovolumetric relaxation time (IVRT) and myocardial performance index (MPI) that are parameters from Systolic and global functional of the heart consecutively, adaptation that we did not see in trained group **(Task 2; Subtask 1).** Additionally, trained group appears to respond more to

dobutamine stress test when compared to sedentary group to increases of heart rate (Figure 11).

- The therapeutic efficacy of Etanercept, Mifepristone alone and combination, was investigated in a murine model of Gulf War Illness by Morris group. Therapeutic Trials were performed in Male C57BL/6J mice (7-8 weeks) pretreated with corticosterone and injected with diisopropyl fluorophosphate (DFP), followed with the drug tests with Etanercept and Mifepristone.

  Treated Groups (n=10):
  Group 1 (Control): No treatment.
  Group 2 (DFP): GWI model (DFP exposed)
  Group 3 (DFP + ENBREL therapy): This group was exposed to DFP. Additionally, we administrated a Monotherapy with Etanercept (ENBREL®).
  Group 4 (DFP + Mifepristone therapy): This group was exposed to DFP. Additionally, we administrated a Monotherapy of Mifepristone (MIFEPREX®).
  Group 5 (DFP + Combination therapy): This group was exposed to DFP. Additionally, we administrated a combined therapy with Etanercept (ENBREL®) + Mifepristone (MIFEPREX®).
  Echo was performed to assess cardiac structure and function before (baseline), after DFP exposure, and after therapy (treatment with Enbrel and Mifepristone). The low dose of DFP exposure produced left ventricular dilation; specifically, LV dilation of end systolic area (ESA) (Figure 13A). The treatment with Enbrel, mifepristone alone or the combination of treatments produced reduction in ESA. The treatment ameliorated the toxic change produced by DFP (Figure 13A). This decrease was not seen in DFP exposed group. End diastolic area (EDA) was not altered in DFP exposed mice. However, the combine treatment (Enbrel and Mifepristone) resulted in enhanced dilation (Figure 13B).

  Systolic function, evaluated by ejection fraction (Figure 13C) was reduced in all DFP groups after exposure. However, these final values were still lower in DFP group and DFP+ENBREL when compared to control. Differences that were not observed in Mifepristone or combination group in relation to the control group. Fraction Shortening (FS) was reduced after DFP exposure (Figure 13D) as compared to control and baseline. Final echocardiographic evaluation (after drug intervention) shows enhanced shortening, revealing the efficacy of the drug. Isovolumetric relaxation time (IVRT) was altered only with combination drug therapy showing increased IVRT when compared to all other groups at the end of the protocol as well as their initial and intermediated assessments (Figure 13E). Figure 13F shows that cardiac output was decreased after DFP exposure compared to control group and baseline. After drug treatment CO was increased as compared to DFP group, suggesting recovery of function. There was no significant alteration observed in left ventricular mass and E/A ratio among the groups (Figure 13G).

  Myocardial Performance Index (MPI) was obtained from Doppler for mitral flow and calculated using ejection time, isovolumetric contraction and relaxation time. MPI which is the ratio of total activity (index of cardiac overload) was significantly increased in DFP exposure mice and re-established in all groups that received therapeutic trial. DFP group was the only one that didn’t re-establish their baseline values (Figure 13H). **(Task 2; Subtask 1)**

- Morris group obtained isovolumetric relaxation time (IVRT) from Doppler. ECHO was significantly lengthened during dobutamine testing in the DFP exposure mice. Nevertheless, after drug treatment, the mifepristone group and combination group showed reduced IVRT during dobutamine test. No significant changes were observed on Enbrel group (Figure 14A). Hemodynamic measures of pump function as ejection fraction on dobutamine stress test showed that all groups that received DFP decreased EF% after this exposure. However, Mifepristone group and combination group (Enbrel+Mifepristone) reestablished their normal values as observed in the baseline of this study (Figure 14B). **(Task 2; Subtask 1)**
- Morris group collected data showing a significant increase in LF/HF ratio after DFP: C= 0.43 (25th percentile = 0.23; 75th percentile = 1.11) vs DFP = 1.13 (25th percentile = 0.73; 75th percentile = 1.76) (Figure 12). This comportment is due to a significant increase of sympathetic modulation (C LFnu = 29.5% vs DFP LFnu = 52%) and a significant decrease of parasympathetic modulation (C HFnu = 70.5% vs DFP HFnu = 48%) after DFP exposure (Figures 11). Drug treatment did not reverse these autonomic effects from DFP exposure: DFP+Enbrel LFnu = 50% and DFP+Enbrel HFnu = 50%; DFP+MIF LFnu = 52% and DFP+MIF HFnu = 48%; DFP+ENB+MIF LFnu = 56.5% and DFP+ENB+MIF HFnu = 43.5% (Figures 15). **(Task 2; Subtask 1)**

What opportunities for training and professional development has the project provided?

- All of the personnel at the VA animal lab have been trained to use all the equipment for the study (electrocardiogram machine, mass spectrometer, etc.) as well as the procedures for the animal protocols. Faculty, staff, and students are encouraged to present their work at local and national meetings and attend consortium investigator’s meetings. There are also continuing opportunities for training.
- The consortium hosted preconference on our team's progress in GWI and ME/CFS at NSU: we had 1000 attendees between the webcast and onsite registration! On site included the policy making program officers at the DoD, NIH, VA and private foundations and 150 scientists, clinicians and advocates. Around 858 logged in to the weblink available for viewing for no charge. GWI veterans approached the GWI consortium and provided valuable input. 3 national stories underway by reporters who attended the conference. DoD program officer's (Dr. Lidie) comments:
  "The meeting was excellent and the science stellar. Thank you very much, Nancy, for inviting me to be part of it and to the entire INIM team for being such great hosts!"

How were the results disseminated to communities of interest?

The high research activity yielded important findings which were published and discussed at CF-GWI consortium.

What do you plan to do during the next reporting period to accomplish the goals?

- We will begin IRB and DoD approvals for human subjects in year 3 based on predictions from the animal studies.
- CDC will continue to evaluate the Enbrel + Mifepristone combined treatment in longer-term exposure conditions. As well as, evaluate/re-evaluate other treatments put forth by the computational group (i.e. minocycline, tacrolimus, etc.).
- Morris group will continue to test cholinergic toxins in mice with examination of peripheral autonomic and cardiac function in combination with acute and chronic exercise. Additionally, we will also continue to evaluate the Enbrel + Mifepristone treatment in GWI model.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Within the last year, critical methods were developed and experiments were completed. This has significant impact on the total project. An important addition was the organization and implementation of an international meeting focused on CF and GWI. We anticipate that the impact will be even greater in the coming years.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS

Changes in approach and reasons for change

No major changes to report

Actual or anticipated problems or delays and actions or plans to resolve them

The CDC group has experienced some delay related to approval of the animal forms. This should be alleviated within the month.

Changes that had a significant impact on expenditures

No major changes to report

Significant changes in use or care of human subjects

No major changes to report

Significant changes in use or care of vertebrate animals
No major changes to report

Significant changes in use of biohazards and/or select agents
No major changes to report

PRODUCTS

Publications, conference papers, and presentations

Journal Publications.

a) O'Callaghan JP, Kelly KA, Locker AR, Miller DB, Lasley SM. 2015. Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of Gulf War Illness. J Neurochem 133(5):708-721.
b) Rice M, del Rosario R, Craddock TJA, Barnes ZM, Klimas NG, Fletcher MA, Zysman J, Broderick G. Gulf War Illness: Is There Lasting Damage to Endocrine-immune Circuitry? Sys Biomed 2016, 2(4): 80-89.
c) Travis J.A. Craddock, Jeanna M. Harvey, Lubov Nathanson, Zachary M. Barnes, Nancy G. Klimas, Mary Ann Fletcher and Gordon Broderick. Using gene expression signatures to identify novel treatment strategies in gulf war illness. BMC Medical Genomics20158:36.
d) O'Callaghan JP, Michalovicz LT, Kelly KA. 2016. Supporting a Neuroimmune Basis of Gulf War Illness. EBioMedicine. http://dx.doi.org/10.1016/j.ebiom.2016.10.037.

Books or other non-periodical, one-time publications.

Other Publications, conference papers, and presentations.

1. Vashishtha S, Russell L, Michalovicz L, Kelley KA, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M, Broderick G. Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model. Int Soc for Traumatic Stress Studies (ISTSS)/ Canadian Inst for Military Veteran's Health Research (CIMVHR) Meeting, Traumatic Stress Research: Enabling Bedside Implementation, Toronto, Canada, May 9-10, 2016; Poster session.
2. Craddock, T. Using genomic drug repurposing and docking to predict novel treatment avenues for Gulf War Illness. Advancing Computational Biology Symposium at Howard University in Washington, DC.
3. Toole JT, Rice MA jr., Nierenberg B, Craddock TJA, Fletcher MA, Klimas NG, Zysman J, Morris M, Broderick G. Increasing Resilience to Traumatic Events: Simulating the Protective Role of Well-Being. Int Soc for Traumatic Stress Studies (ISTSS)/ Canadian Inst for Military Veteran's Health Research (CIMVHR) Meeting, Traumatic Stress Research: Enabling Bedside Implementation, Toronto, Canada, May 9-10, 2016; Poster session.

4. Machi JF, Conti F, Hernandez D, Salgueiro L, Klimas N, Fletcher MA, Morris M. Low-Intensity Exercise Training Improves Autonomic Modulation and Cardiac Function in a Potential Model of Gulf War Illness in mice with Organophosphate (DFP) Exposure: Analyze by Noninvasive Method of Cardiovascular Measures. Nova Southeastern Univ. and Miami VA Healthcare Syst. EB 2016. Poster session.
5. Broderick G, Vashishtha S, Russell L, Michalovicz L, Kelley KA, Vrana JA, Locker AR, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller D, O'Callaghan J, Morris M. Stress Potentiation of the Brain's Immune Response to Neurotoxic Exposure in the Field: An Animal Model of Gulf War Illness. IACFS Annual Meeting in October, 2016 in Fort Lauderdale, FL. Poster session
6. Toole JT, Rice, MA Jr., Cargill J, Craddock TJA, Nierenberg B, Klimas NG, Fletcher MA, Morris M, Zysman J, Broderick G. Increasing Resilience to Traumatic Stress: Understanding the Protective Role of Well-Being. IACFS Annual Meeting in October, 2016 in Fort Lauderdale, FL. Poster session
7. Craddock TJA, Harvey JM, Nathanson L, Barnes ZM, Klimas NG, Fletcher MA, Broderick G. Using gene expression signatures to identify novel treatment strategies in gulf war illness. IACFS Annual Meeting in October, 2016 in Fort Lauderdale, FL. Poster session
8. Jaundoo R, Bohmann J, Gutierez G, McDonough J, Klimas NG, Broderick G, Morris M, Craddock TJA. Structure-Based Repurposing of FDA-Approved Drugs to Identify Specific Small Molecule Inhibitors of TNF-alpha, IL-2, and the Glucocorticoid Receptor for Treatment of Gulf War Illness. IACFS Annual Meeting in October, 2016 in Fort Lauderdale, FL. Poster session
9. Cournoyer J, Broderick G, Collado F, Fletcher MA, Klimas NG. The Use of the Respiratory Exchange Ratio in Assessing the Metabolic Efficiency of Patients with ME/CFS and GWI. IACFS Annual Meeting in October, 2016 in Fort Lauderdale, FL. Poster session
10. O'Callaghan JP, Harry GJ, McPherson CA, Heijnen JJ, Michalovicz LT, Klimas NG. (2017) Scientific Symposium: Chemically-induced neuroinflammation and "sickness behavior" disorders. 56th Annual Meeting of the Society of Toxicology.
11. Michalovicz L.T., Kelly K.A., Locker A.R., Vrana J.A., Broderick G., Miller D.B., O'Callaghan J.P. (2016) Using novel ALDH1L1 bacTRAP technology to evaluate the astrocyte-specific responses to Gulf War Illness-related exposures. 46th Annual Meeting of the Society for Neuroscience.
12. Kelly K.A., Locker A.R., Michalovicz L.T., Vrana J.A., Vashishtha S., Broderick G., Miller D.B., O'Callaghan J.P. (2016) A mouse model of Gulf War Illness reveals a primed neuroinflammatory response to subsequent systemic inflammatory challenge. 46th Annual Meeting of the Society for Neuroscience.
13. Locker A.R., Kelly K.A., Michalovicz L.T., Vrana J.A., Barnes Z.M., Fletcher M.A., Miller D.B., O'Callaghan J.P. (2016) Corticosterone primes the inflammatory response to Gulf War Illness-associated agent exposures in the brain, but not the periphery. 46th Annual Meeting of the Society for Neuroscience.
14. Vrana J.A., Locker A.R., Kelly K.A., Michalovicz L.T., LeBouf R.F., O'Callaghan J.P., Miller D.B. (2016) Quantification of brain acetylcholine in a mouse model of Gulf War Illness using HILIC-UPLC-MS/MS. 46th Annual Meeting of the Society for Neuroscience.

15. Michalovicz L.T., Kelly K.A., Locker A.R., Miller D.B., O'Callaghan J.P. (2016) Corticosterone priming of the neuroinflammatory response to AChE inhibitors results in overexpression of TLR2 and downstream targets, but not activation of the NLRP3 inflammasome. 55th Annual Meeting of the Society of Toxicology.
16. Kelly K.A., Locker A.R., Michalovicz L.T., Miller D.B., O'Callaghan J.P. (2016) Exploration of the Gulf War Illness phenotype in a mouse model challenged with LPS at long term time points. 55th Annual Meeting of the Society of Toxicology.
17. Locker A.R., Kelly K.A., Michalovicz L.T., Miller D.B., O'Callaghan J.P. (2016) Organophosphate-induced neuroinflammation, with and without corticosterone pretreatment, is not due to acetylcholinesterase inhibition. 55th Annual Meeting of the Society of Toxicology.
18. Michalovicz L.T., Locker A.R., Kelly K.A., Miller D.B., O'Callaghan J.P. (2015) Chronic corticosterone primes the brain response to select neuroinflammatory agents by overexpression of Toll-like receptor 2 and S100A8: a potential role for microglia. 45th Annual Meeting of the Society for Neuroscience.
19. Locker A.R., Kelly K.A., Michalovicz L.T., Miller D.B., O'Callaghan J.P. (2015) Corticosterone primes the neuroinflammatory responses to Gulf War Illness associated exposures: Effects of irreversible vs. reversible acetylcholinesterase inhibitors. 45th Annual Meeting of the Society for Neuroscience.
20. O'Callaghan J.P., Michalovicz L.T., Kelly K.A. Supporting a Neuroimmune Basis of Gulf War Illness. Commentary. EBioMedicine (In press).
21. MACHI, JF; CONTI, F; HERNANDEZ, D; SALGUEIRO, L; SCHIMDT R; MORRIS, M. Institute for Neuro-Immune Medicine, College of Osteopathic Medicine, Nova Southeastern University, Ft. Lauderdale, FL; Miami Veterans Affairs Healthcare System, Miami, FL. Cardiac Function in a Murine Model of Gulf War Illness (GWI): Combination of Organophosphate (DFP) and Exercise Training. IACFS/ME Conference 2016.
22. MACHI, JF; ALBUQUERQUE, O; FREITAS, S.C. ;CRUZ, P.L.; BARBOZA, C.A.B. ; MORRIS, M.; ANGELIS, K.; IROGOYEN; M.C.I. Nova Southeastern University, Institute of Neuro Immune Medicine Fort Lauderdale United States of America, Heart Institute (InCor) -FMUSP, Department Hypertension Unit Sao Paulo Brazil. Aerobic exercise training prevents autonomic impairment, oxidative stress and systemic inflammation in a model of aging, menopause e metabolic syndrome, European Heart Journal 37 (Abstract Supplement), 133, Italy – Rome: 2016.
23. MACHI, J.F.; CONTI, F.; HERNANDEZ, D; SALGUEIRO, L; KLIMAS, N; FLETCHER, M; MORRIS, M. Low-Intensity Exercise Training Improves Autonomic Modulation and Cardiac Function in a Potential Model of Gulf War Illness In mice with Organophosphate (DFP) Exposure: Analyze by Noninvasive Method of Cardiovascular Measures. In: EXPERIMENTAL BIOLOGY 2016, 2016, San Diego. EB 2016, 2016.
24. MACHI, J.F.; Bernardes, N; FREITAS, S.; CRUZ, P.; NASCIMENTO, A.; MORRIS, M.; DE ANGELIS, K.; IRIGOYEN, M. C. Aging and menopause are associated with autonomic dysfunction and systemic inflammation. In: 42nd Annual Eastern-Atlantic Student Research Forum, 2016, Miami. 42nd Annual Eastern-Atlantic Student Research Forum, 2016.

25. MACHI, J. F.; SALGUEIRO, L.; SCHMIDT, R.; FLETCHER, M.; KLIMAS, M.; BRODERICK, G.; MORRIS, M. Cardiac Function in a Murine Model of Gulf War Illness (GWI): Success in Therapeutic Trial. 56TH ANNUAL MEETING, Baltimore, MD March 13-17, 2017.
26. MACHI, J. F; Bernardes, N; CRUZ, P; SILVA, I. M; BARBOSA, M; NASCIMENTO, A; DE ANGELIS, K; MORRIS, M**;** IRIGOYEN, M. C. Exercise training prevents diastolic dysfunction induced by fructose overload in old female ovariectomized rats. 2015. ESC CONGRESS. UNITED KINGDOM, London. 2015.

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products
Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Mariana Morris, PhD
Project Role:	PI
Research Identifier:	eCommons: mariana
Nearest person month worked:	3
Contribution to Project:	Overseeing the entire research project. Established the animal protocols and in charge of the animal research. Oversees hiring of all personnel.
Funding Support:	NIH

Name:	Gordon Broderick, PhD
Project Role:	Co-Director
Research Identifier:	eCommons: gbroderick
Nearest person month worked:	3
Contribution to Project:	Head of computational biology. Has worked on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	NIH, VA

Name:	Travis Craddock, PhD

Project Role:	Co-Investigator
Research Identifier:	eCommons: TRAVISCRADDOCK
Nearest person month worked:	3
Contribution to Project:	Has worked on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	NIH, CFIDS Association of America, Nova Southeastern University PFRDG

Name:	Nancy Klimas, MD
Project Role:	Co-Director
Research Identifier:	eCommons: nklimas
Nearest person month worked:	1
Contribution to Project:	Head of clinical sciences. Reviewed modeling from th computational biology team in regards to human subjects to help establish protocols.
Funding Support:	NIH, VA, CDC

Name:	Mary Ann Fletcher, PhD
Project Role:	Co-Investigator
Research Identifier:	eCommons: mfletche
Nearest person month worked:	2
Contribution to Project:	Director of the immunology core.
Funding Support:	NIH, VA

Name:	James Blount
Project Role:	Administrative Coordinator
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Monitored budget, maintained meeting schedules, prepared quarterly and annual reports, assisted in establishing sub-awards, and other duties associated with administration of the award.
Funding Support:	No longer funded by this grant

Name:	Amanpreet Cheema, PhD
Project Role:	GWI program administrator
Research Identifier:	None
Nearest person month worked:	4
Contribution to Project:	Monitored budget, maintained meeting schedules, prepared quarterly and annual reports, assisted in establishing sub-awards, and other duties associated with administration of the award.
Funding Support:	None

Name:	Ana Del Alamo
Project Role:	Research Associate
Research Identifier:	None
Nearest person month worked:	10
Contribution to Project:	Assisted Dr. Klimas in in her work concerning the human subject protocols.
Funding Support:	No longer funded by this grant

Name:	Diana Hernandez, PhD
Project Role:	Research Associate
Research Identifier:	None
Nearest person month worked:	4
Contribution to Project:	Active in animal experiments
Funding Support:	No longer funded by this grant

Name:	Jacqueline Machi, PhD
Project Role:	Research Assistant III
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Luis Salguiero, PhD
Project Role:	Laboratory Specialist
Research Identifier:	None
Nearest person month worked:	7
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Rodrigo Schmidt
Project Role:	Research Assistant I
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Active in animal experiments
Funding Support:	None

Name:	Mark Rice
Project Role:	Data Control Specialist
Research Identifier:	None
Nearest person month worked:	9
Contribution to Project:	In charge of the data analysis and has assisted on the computational models for animal and human research to assist in protocols and findings.

Funding Support:	No longer funded by this grant

Name:	Shane Hills
Project Role:	Data Control Specialist
Research Identifier:	None
Nearest person month worked:	3
Contribution to Project:	In charge of the data analysis and has assisted on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	No longer funded by this grant

Name:	Hooman Sedghamiz
Project Role:	Data Control Specialist
Research Identifier:	None
Nearest person month worked:	2
Contribution to Project:	In charge of the data analysis and has assisted on the computational models for animal and human research to assist in protocols and findings.
Funding Support:	None

Name:	Jonathan Toole
Project Role:	Research Assistant
Research Identifier:	None
Nearest person month worked:	12
Contribution to Project:	Has assisted on the computational models for animal and human research to assist in protocols and findings
Funding Support:	None

Name:	Filipe Conti
Project Role:	Visiting Scholar/Graduate Student
Research Identifier:	None
Nearest person month worked:	6
Contribution to Project:	Active in animal experiments
Funding Support:	CAPES (Brazilian government agency) Not funded by this grant

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Craddock was awarded the following CDMRP grants:
1) GW150199 (Craddock)
Improving Diagnostics and Treatments for GWI Females by Accounting for the Effects of PTSD
2) GW150144 (Craddock)

Disentangling the Effects of PTSD from GWI for Improved Diagnostics and Treatments.
Dr. Broderick has 10% salary commitment on each of these.
Start of the performance period: Oct. 01, 2016

Stress Hormone Enhancement of OP-Induced Neuroinflammation as an Animal Model of GWI: The Role of Toll-Like Receptors and Plasticity. S.M. Lasley (PI); J.P. O'Callaghan (Co-I); K.A. Kelly (Co-I); D.B. Miller (Co-I). The goal of this research is to expand upon the previous work that found DFP to cause significant neuroinflammation that was exacerbated by pretreatment with the stress hormone, corticosterone (CORT). We will extend our mouse model to additional GWI-relevant organophosphate (OP) exposures, identify molecular pathways underlying CORT "priming" of OP-related neuroinflammation to reveal potential therapies, define functional measures that reflect downstream CNS effects of CORT priming, and, demonstrate the efficacy of therapeutic interventions after neuroinflammation develops. (CDMRP 2017-2020)

Therapeutic Intervention of Glial-Mediated Enhancment of Neuroinflammation in an Established Model of GWI. K.A. Kelly (PI); J.P. O'Callaghan (Co-I). The goal of this research is to characterize the contribution of high physiological stress and glial cell type on the GWI phenotype in the established CORT-primed DFP mouse model of GWI and to test the therapeutic potential of FDA-approved inflammatory and cell-type specific inhibitors. (CDMRP 2017-2019)

Stress Hormones Affect the Neuroinflammosome and Neurotoxicity. J.O. Callaghan (PI); D.B. Miller (Co-I). The goal of this research is to characterize early and sensitive biomarkers of neurotoxicity and neuroinflammation. (CDC intramural funding NORA 2017-2021)

What other organizations were involved as partners?

Name:	Centers for Disease Control and Prevention National Institute for Occupational Safety and Health
Location:	1095 Willowdale Road Morgantown, WV 26505
Contribution:	Chemical toxicology project collaboration
Financial:	None
In-kind Support:	None
Facilities:	None
Collaboration:	Partner's staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None

Name:	Southwest Research Institute
Location:	5220 Culebra Road, PO Drawer 28510 San Antonio, TX 78228
Contribution:	Assisting on drug choices to test in animals and humans.

Financial:	None
In-kind Support:	None
Facilities:	None
Collaboration:	Partner’s staff works with project staff in the project.
Personnel Exchanges:	None
Other:	None

Name:	South Florida VA Foundation for Research & Education Inc.
Location:	1201 NW 16th Street, Room #2A103 Miami, FL 33125
Contribution:	Providing subjects and space for human trials in future. Help with establishing human protocols.
Financial:	None
In-kind Support:	None
Facilities:	Project staff uses the partner’s facilities for project activities.
Collaboration:	Partner’s staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other’s facilities. Dr. Klimas and Dr. Fletcher are on staff at Nova Southeastern University and the Miami VA.
Other:	None

Name:	South Florida VA Foundation for Research & Education Inc. – Animal Facility
Location:	1201 NW 16th Street, Room #2A102 Miami, FL 33125
Contribution:	
Financial:	None
In-kind Support:	None
Facilities:	Project staff uses the partner’s facilities for project activities.
Collaboration:	Partner’s staff works with project staff in the project.
Personnel Exchanges:	Project staff uses each other’s facilities. Dr. Morris is on staff at Nova Southeastern University and the Miami VA.
Other:	None

SPECIAL REPORTING REQUIREMENTS

Collaborative Awards:
Nothing to Report

Quad Charts (please next page)

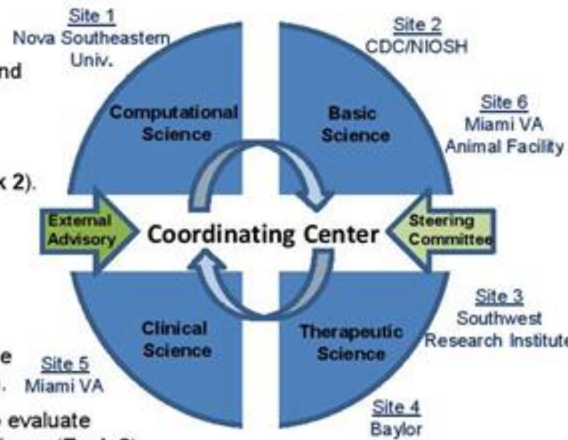
Understanding Gulf War Illness: An Integrative Modeling Approach

Award Number: GW120045 / W81XWH-13-2-0085

PI: Dr. Mariana Morris **Org:** Nova Southeastern University **Award Amount:** $4,102,527

Approach To develop a translational model of GWI for rapid identification of molecular targets and prediction of effective therapeutic interventions. The effectiveness of candidate treatment in terms of system abatement and recovery of regulatory network configuration will be assessed in GWI subjects in phase 1 translational studies.

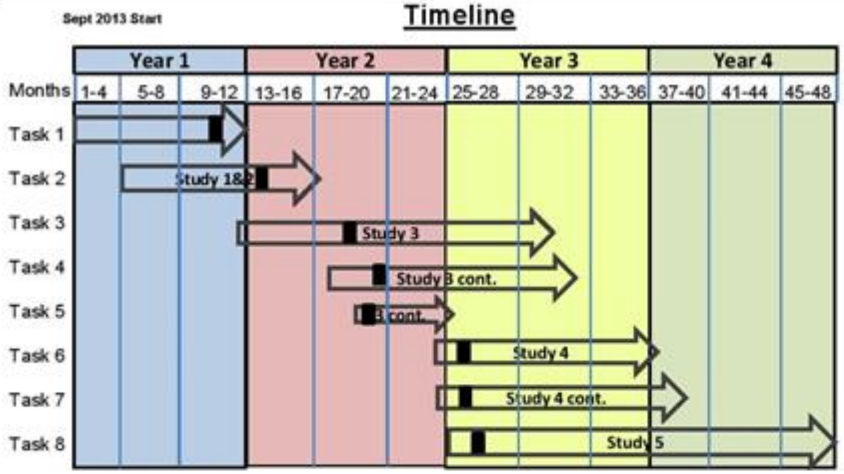
- ❑ ***Study 1:*** Characterize the autonomic neural/adrenal dysfunction in a mouse model of GWI using validation and direction from computational biology (**Task 2**).
- ❑ ***Study 2:*** Characterize the molecular and cellular phenotype of GWI in a mouse model to evaluate the role of stress response in persistence of the illness (**Task 2**).
- ❑ ***Study 3:*** Integrate human (previously completed) and animal studies using computational biology to identify mediators of deregulated balance and test putative therapeutics (**Task 3-5**)
- ❑ ***Study 4:*** Evaluate therapeutics suggested by computational model in GWI animal models. Two or three most favorable will move on to human testing (**Task 6-7**).
- ❑ ***Study 5:*** Perform translational human clinical trials to evaluate homeostasis "reset" as well as preliminary safety and efficacy (**Task 8**).



Accomplishments to date

1-The consortium hosted preconference on our team's progress in GWI and ME/CFS at NSU.
2-Morris group observed autonomic dysfunction in mice model of GWI treated with DFP.
3-Members of GWIC have submitted 11 articles which are under review.
4-Established minimum levels of corticosterone required to maintained a heightened pro-inflammatory response to the sarin surrogate, DFP.
5- Dosing pilots performed for etanercept and mifepristone after approval from Miami VAHCS IACUC.
6-GWIRP featured GWIC progress, especially development of animal models.
7-The human subjects protocol and consent form has been finalized.

Timeline



Goals/Milestones

FY13 Goal – Administrative structure for animal/human studies (Task 1)
- ☑ Kick-off meetings with GWRP staff and study PIs
- ☑ Protocol preparation and initiation of approvals for animal/human use
- ☑ Coordinating center database set-up

FY14 Goal – Studies 1- 3 - Refinement and enhancement of models for GWI
- ☑ Establish model of autonomic dysfunction as a surrogate for GWI (**Task 2**)
- ☑ Identification of Illness specific networks with focus on human and mouse comparisons (**Task 3**)
- ☐ Large-scale simulation of treatment. (**Task 4**)
- ☐ Define/deploy optimization and target intervention possibilities (**Task 5**)

FY15 Goal – Study 4 - Candidate treatment courses
- ☑ Identify candidate treatment courses for GWI (**Task 6**)
- ☑ Select and test therapies in animals (**Task 7**)

FY16 Goal – Study 5 - Perform translational human clinical trials
- ☐ Verify treatment effectiveness in human subjects n=30 (**Task 8**)

APPENDICES

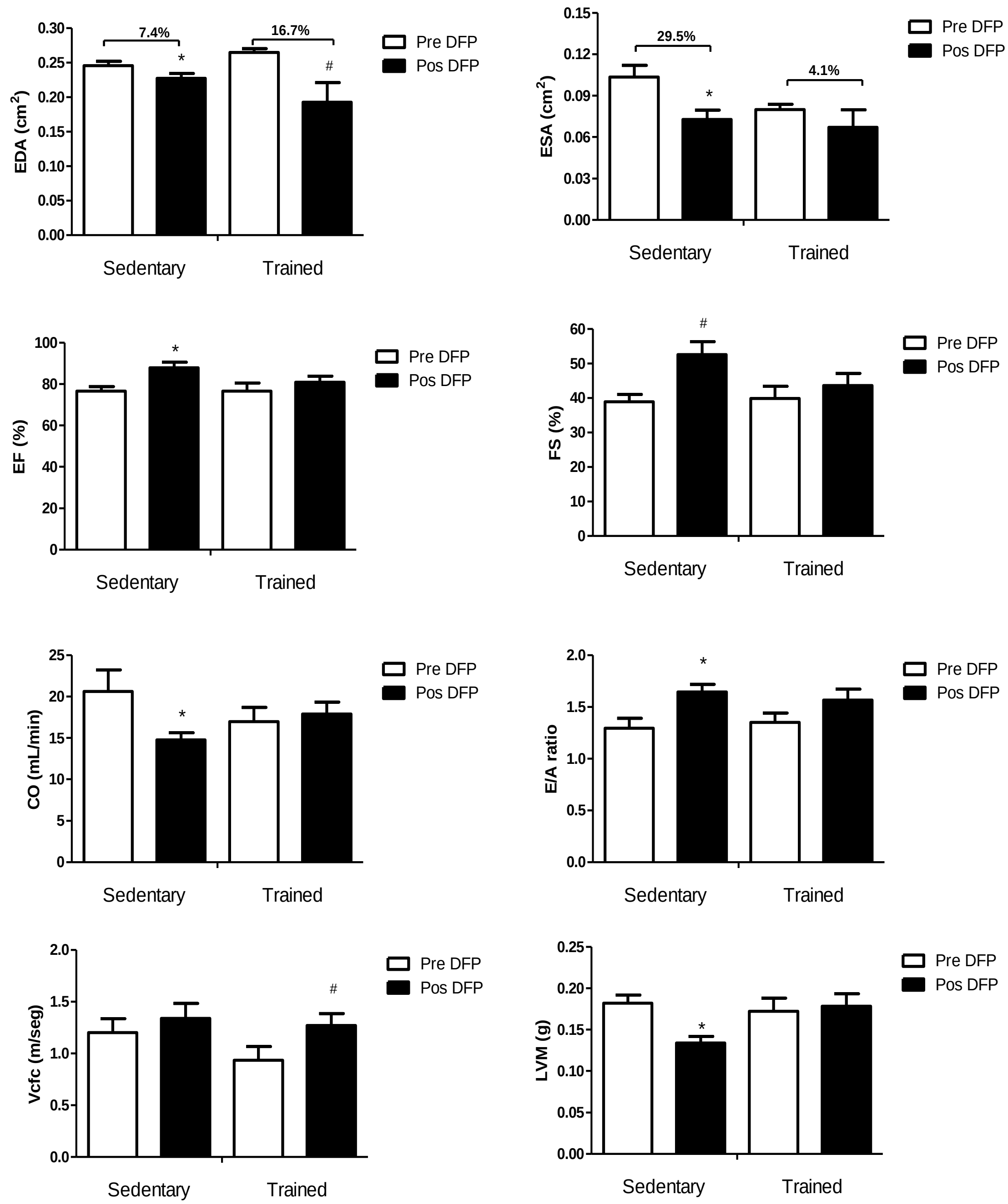


Figure 1: Echocardiography (ECHO) and electrocardiogram (ECG) with dobutamine stress test were performed before and after DFP injection.

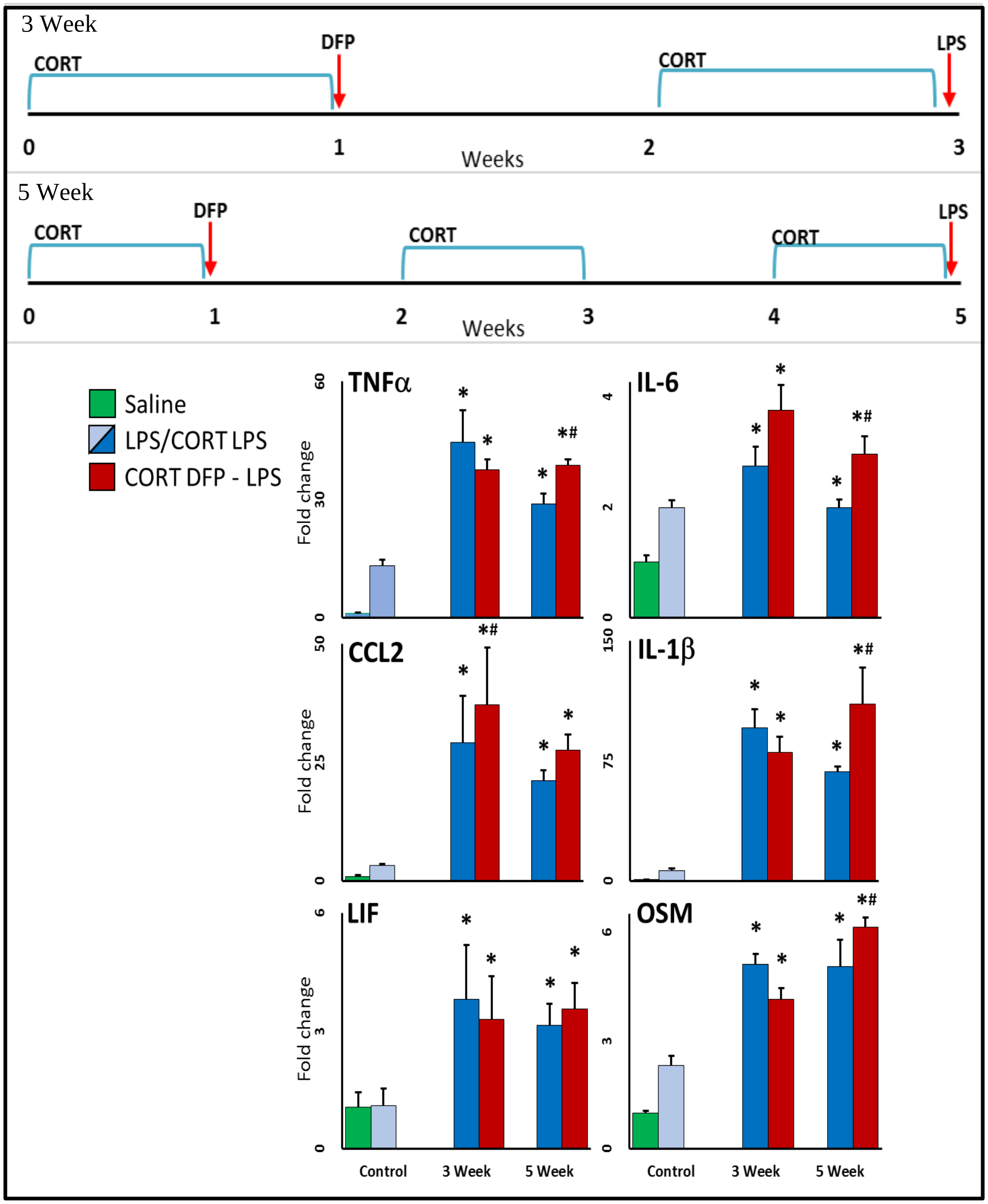


Figure 2: Mice were exposed to 3 week and 5 week long GWI "phenotype exposure" paradigms, where mice received 7 days of CORT every other week for the specified time. Neuroinflammation was evaluated by PCR measurement of relative cytokine mRNA expression 6 hours following LPS treatment. * indicates statistical significance ($p \leq 0.05$) from appropriate control group. # indicates statistical significance ($p \leq 0.05$) within exposure (saline vs. agent).

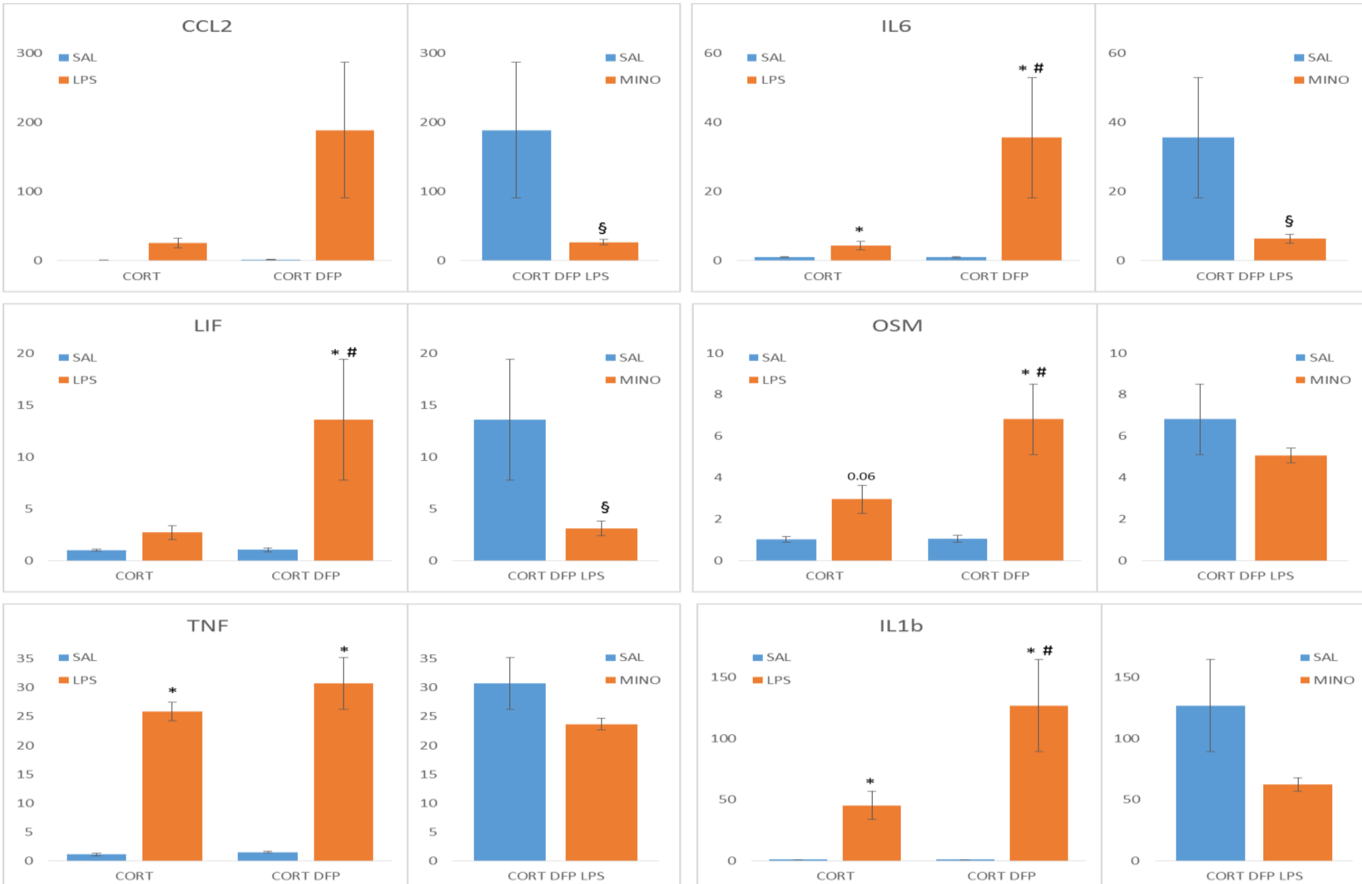


Figure 3: Mice were exposed to our initial GW theater-like conditions (4 days of Cort in drinking water, DFP on day 5) and then given an inflammagen challenge (LPS) 48 h later (on day 7). Mice received either saline or minocycline one hour prior to LPS exposure. Neuroinflammation was evaluated by PCR measurement of relative cytokine mRNA expression 6 hours following LPS treatment. * indicates statistical significance ($p \leq 0.05$) from appropriate control group. # indicates statistical significance ($p \leq 0.05$) within exposure (saline vs. agent). § indicates statistical significance ($p \leq 0.05$) within treatment (saline vs. minocycline).

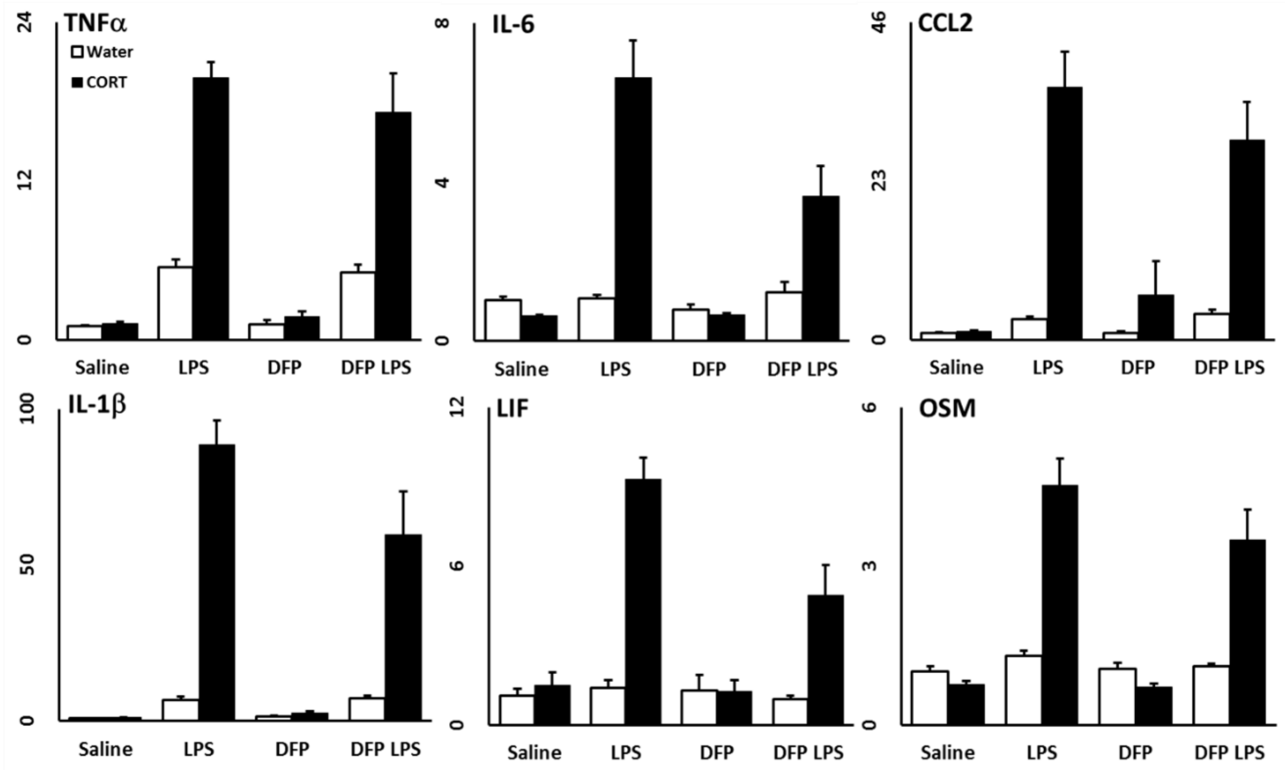


Figure 4: CX3CR1 -/- mice were exposed to a short-term GWI paradigm (7 days of CORT in drinking water, DFP on day 7) and then given an inflammatory challenge (LPS) 48 hours later (on day 9). Neuroinflammation was evaluated by PCR measurement of relative cytokine mRNA expression.

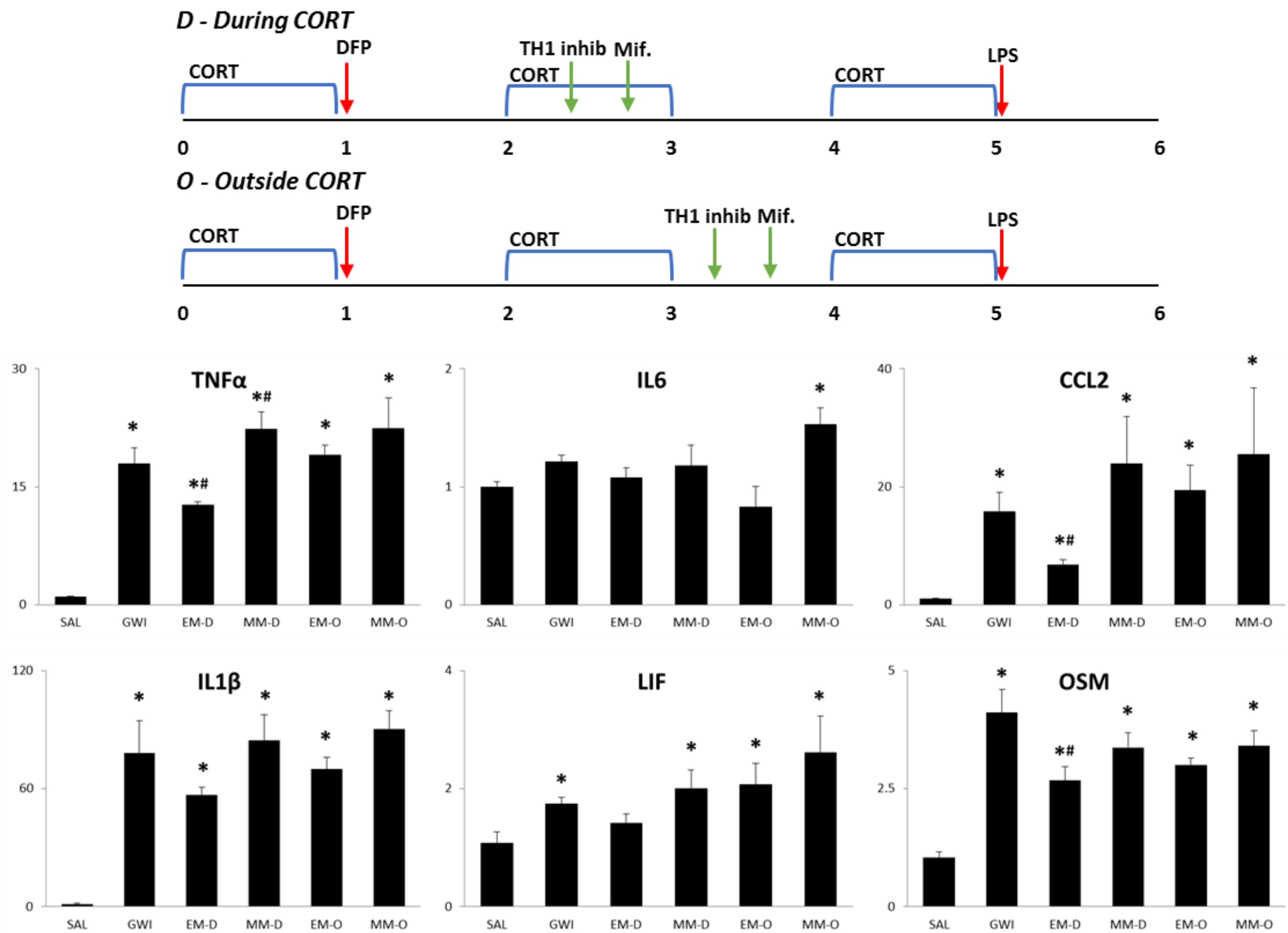


Figure 5: Mice were exposed to our 5 week GWI paradigm and given two different treatment combinations (Enbrel + Mifepristone or Minocycline + Mifepristone) either during or in between CORT exposures. At the end of the 5 week period, mice were given an inflammatory challenge (LPS) and sacrificed 6 hours later. Neuroinflammation was evaluated by PCR measurement of relative cytokine mRNA expression. * indicates statistical significance ($p \le 0.05$) from saline. # indicates statistical significance ($p \le 0.05$) from GWI.

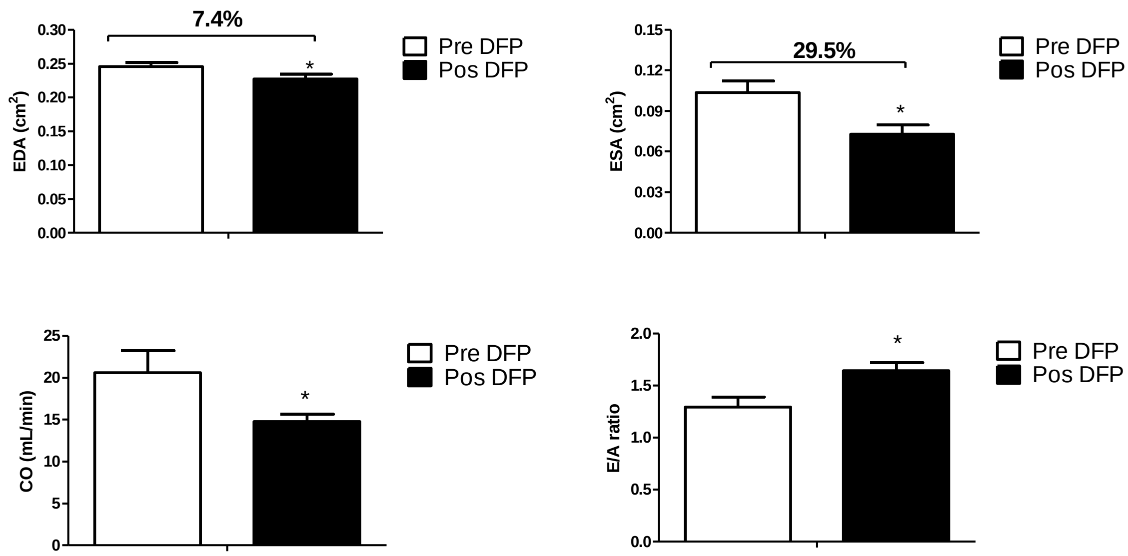


Figure 6: Data are represented as mean ± standard error of the mean. EDA end diastolic area, ESD end systolic area, CO cardiac output and E/A e wave and a wave ratio *vs pre DFP.

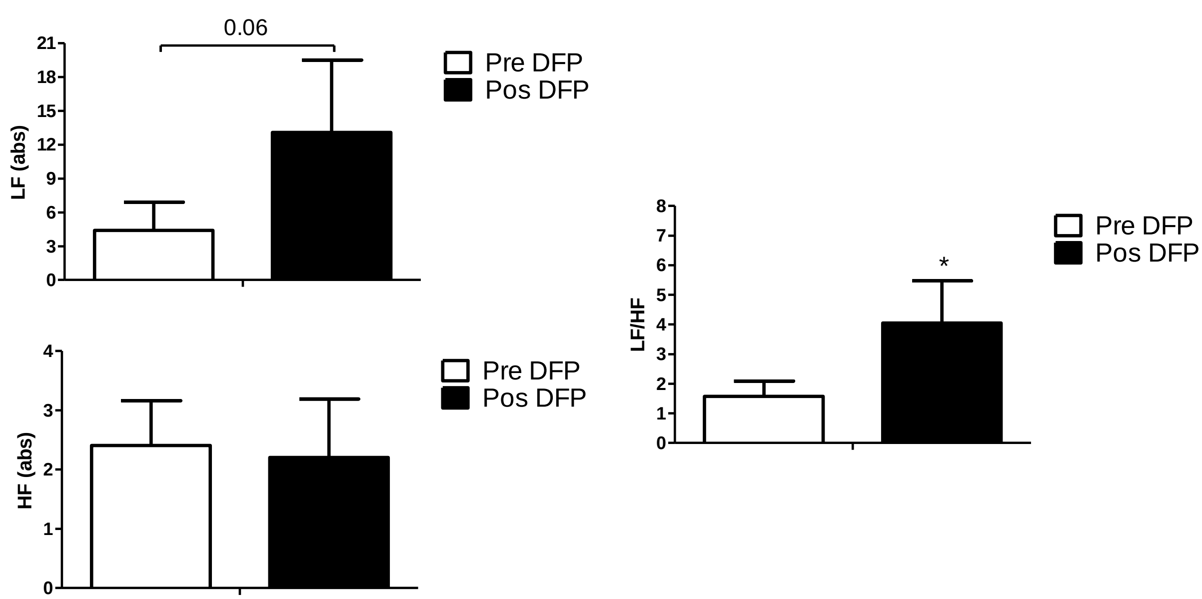


Figure 7: Data are represented as mean ± standard error of the mean. LF abs- Low Frequency Band - sympathetic representative, HF -High Frequency Band - vagal representative, LF/HF- Ratio (sympathetic and parasympathetic balance) *vs pre DFP.

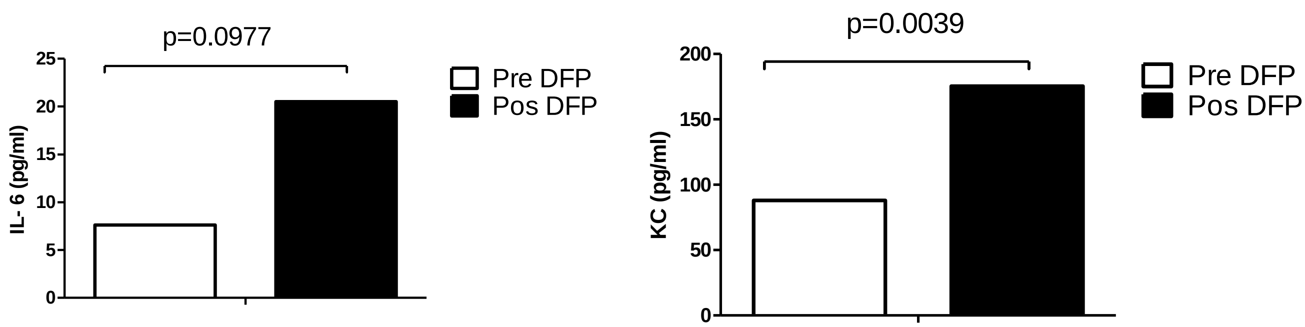


Figure 8: Data are represented as median. Plasma cytokines were measured using ELISA kits, IL6=Interleukin-6, KC= Keratinocyte Chemoattractant chemokine *vs pre DFP.

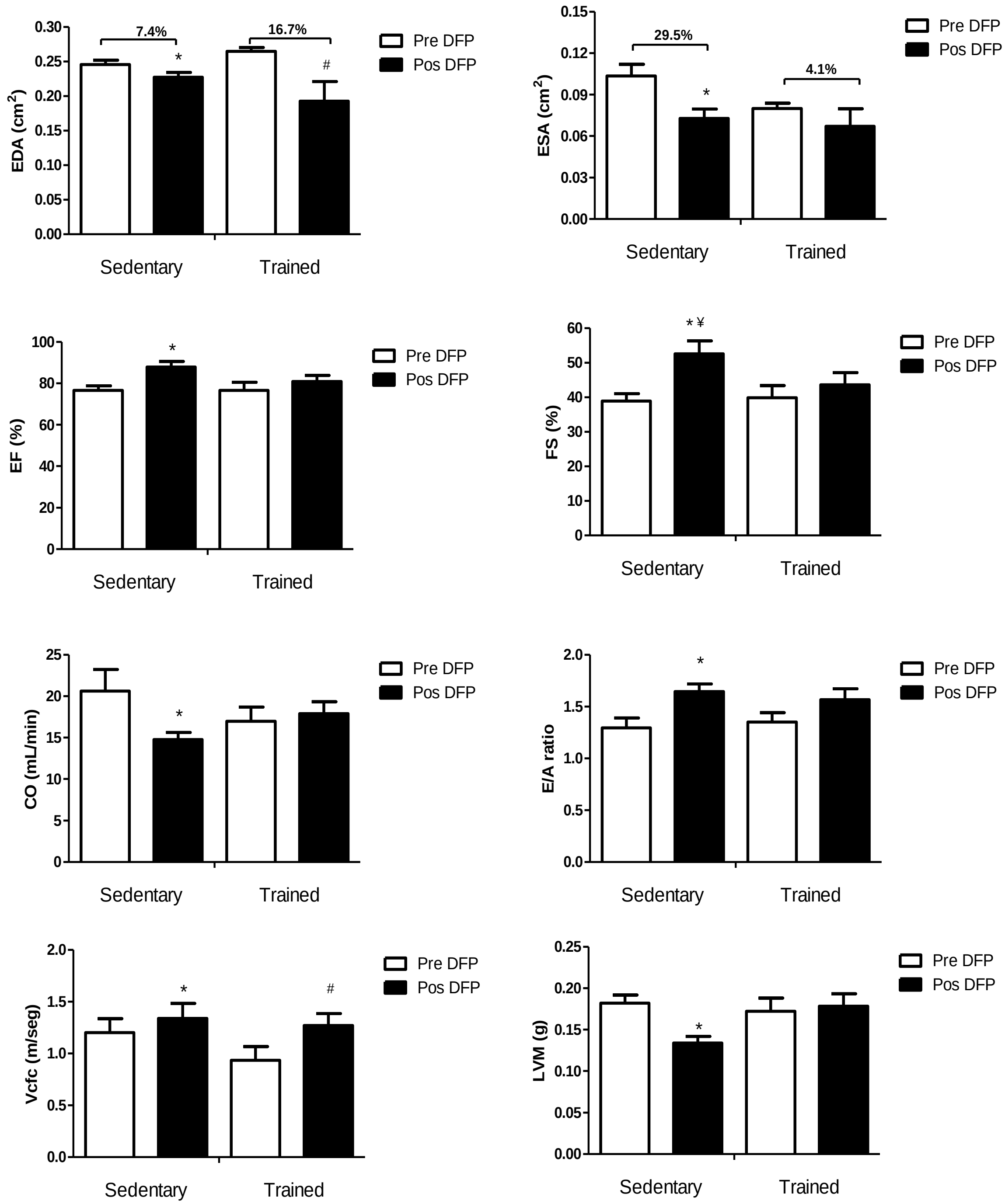


Figure 9: Data are represented as mean ± standard error of the mean. EDD end diastolic diameter, ESD end systolic diameter, EF ejection fraction, FS fraction shortening, CO cardiac output, E/A e wave and a wave ratio, VCFC velocity of circumferential fiber shortening, LVM left ventricular mass. * vs pre DFP same group (Sedentary), # vs pre DFP same group (trained), ¥ vs trained group.

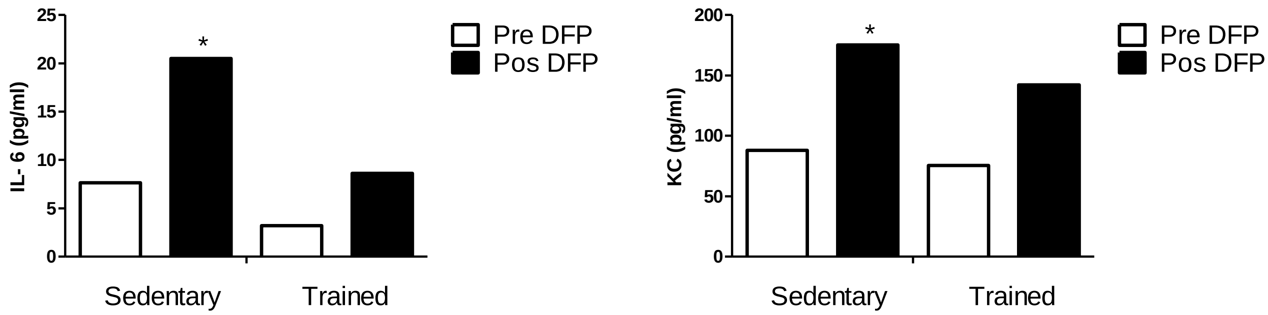


Figure 10: Data are presented as median. Interleukin-6 (IL6-), Keratinocyte Chemoattractant (KC). * vs pre DFP same group.

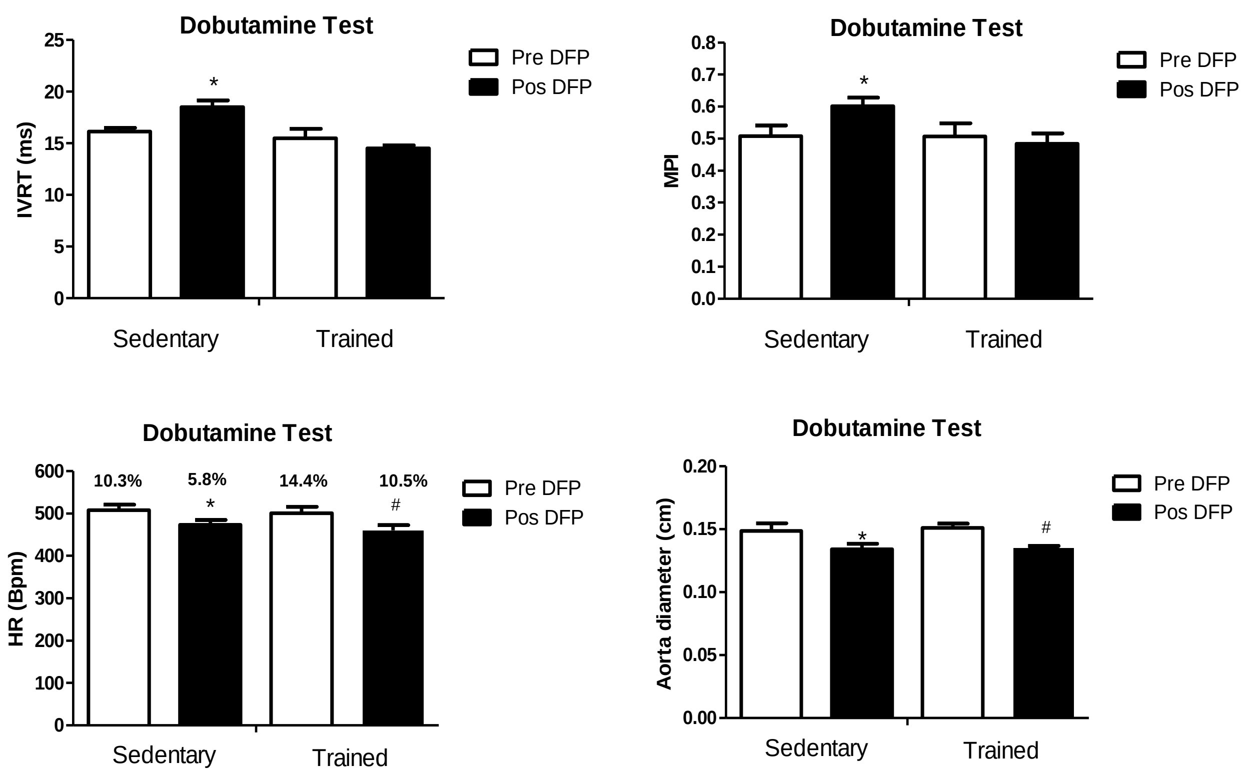


Figure 11: Data are represented as mean ± standard error of the mean. **IVRT** isovolumetric relaxation time, **MPI** myocardial performance index, **HR** heart rate. * vs pre DFP same group (sedentary), # vs pre DFP same group (trained).

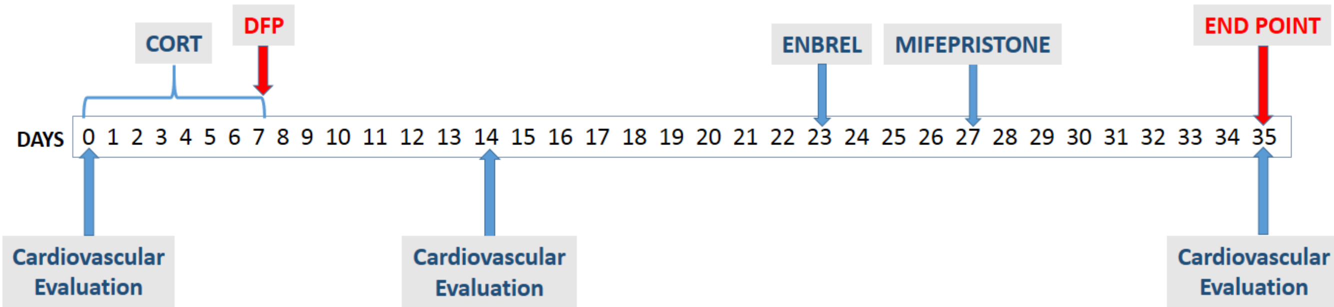


Fig 12: Cardiovascular evaluation was performed in mice with 7-8 weeks' years old as baseline values followed by one week of cortisol (200 mg/Lt in drinking water), followed by a dose of DFP (1.5 mg/kg, s.c) on the last day. One week after the DFP injection, the second time point evaluation was performed. Two weeks and three days after the DFP exposure, Etanercept (Enbrel: 10 mg/Kg, i.p.) is given. Four days following Enbrel treatment, Mifepristone (40 mg/Kg, i.p.) is given. Eight days after mifepristone treatment, the endpoint measurements were taken and mice were sacrificed.

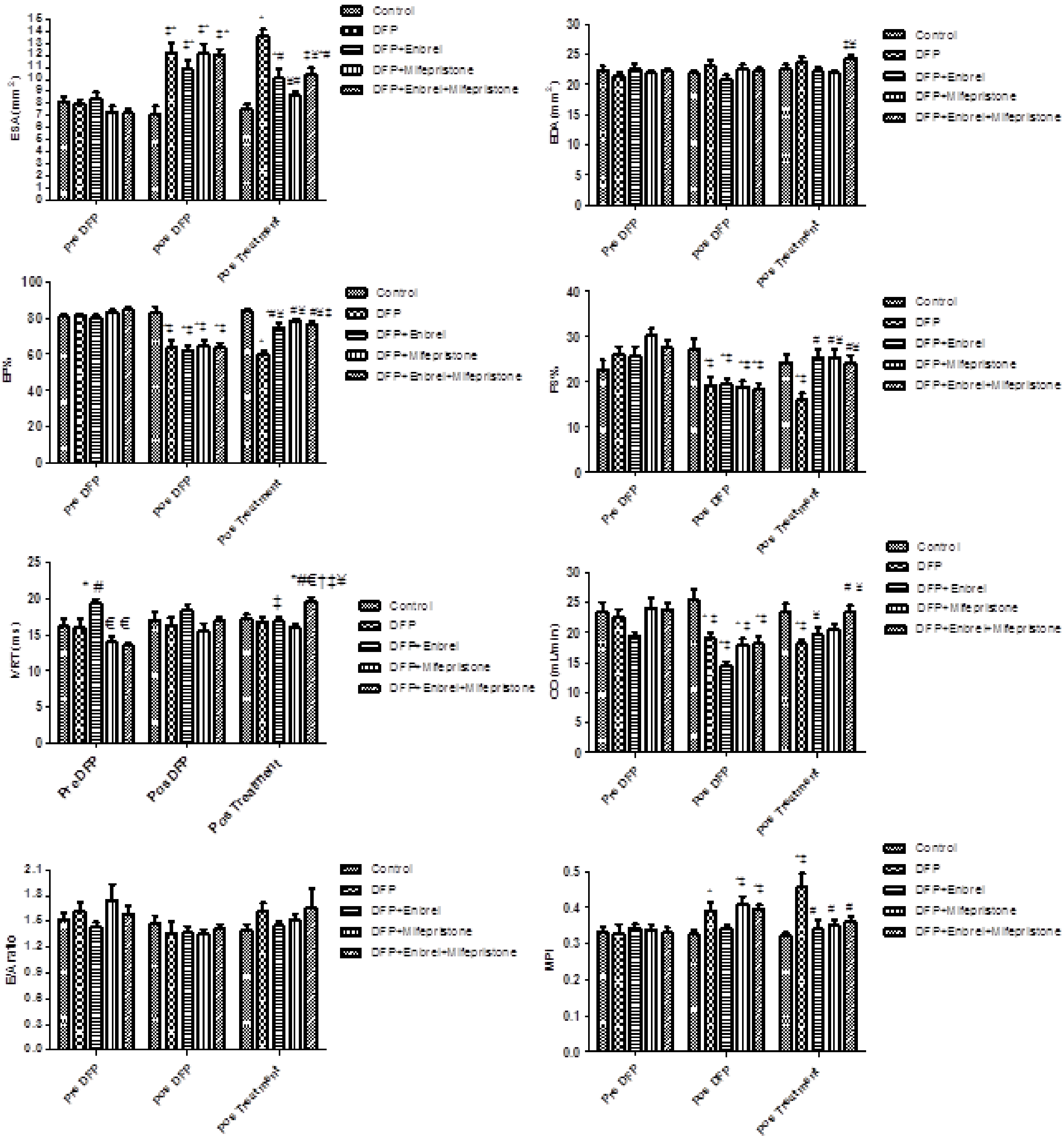


Figure 13: End systolic area (ESA), End Diastolic area (EDA), Ejection Fraction (EF %), Fraction Shortening (FS %), Isovolumetric Relaxation Time (IVRT), Cardiac Output (CO), E wave and A wave ratio (E/A ratio), Myocardial Performance Index (MPI). Data are represented as mean ± standard error of the mean. $p < 0.05$ * versus control, $p < 0.05$ # versus DFP, $p < 0.05$ € versus DFP+ Enbrel, $p < 0.05$ † versus DFP + Mifepristone, $p < 0.05$ ‡ versus initial of the same group, $p < 0.05$ ¥ versus intermediate

of the same group. Data were analyzed using ANOVA and Bonferroni post hoc test (n=10).

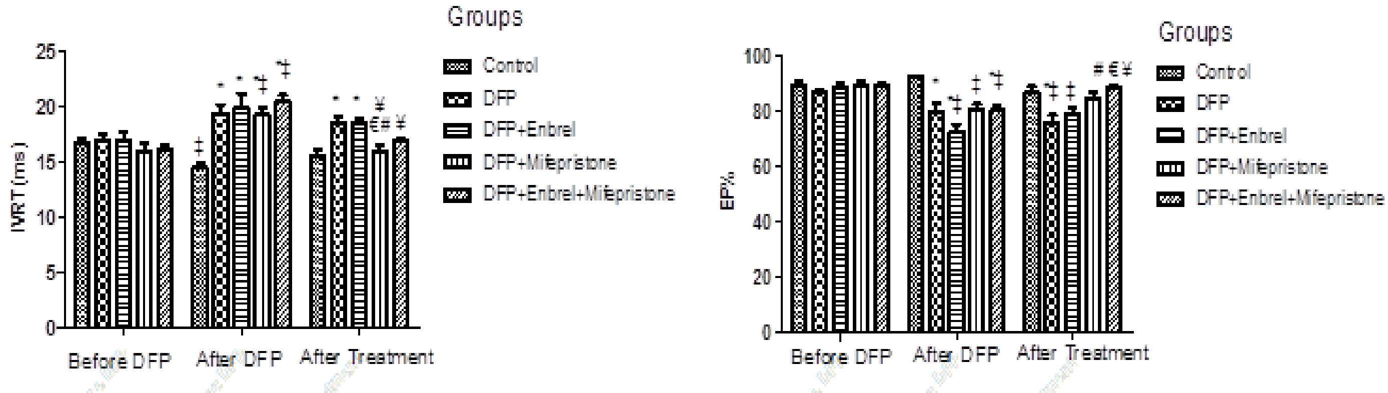


Figure 14: Isovolumetric Relaxation Time (IVRT) and Ejection Fraction (EF%). Data are represented as mean ± standard error of the mean. * p <0.05 versus control, # p <0.05 versus DFP, € <0.05 versus DFP+ Enbrel, ‡ <0.05 versus initial of the same group, ¥ <0.05 versus intermediate of the same group. Data were analyzed using ANOVA and Bonferroni post hoc test (n=10).

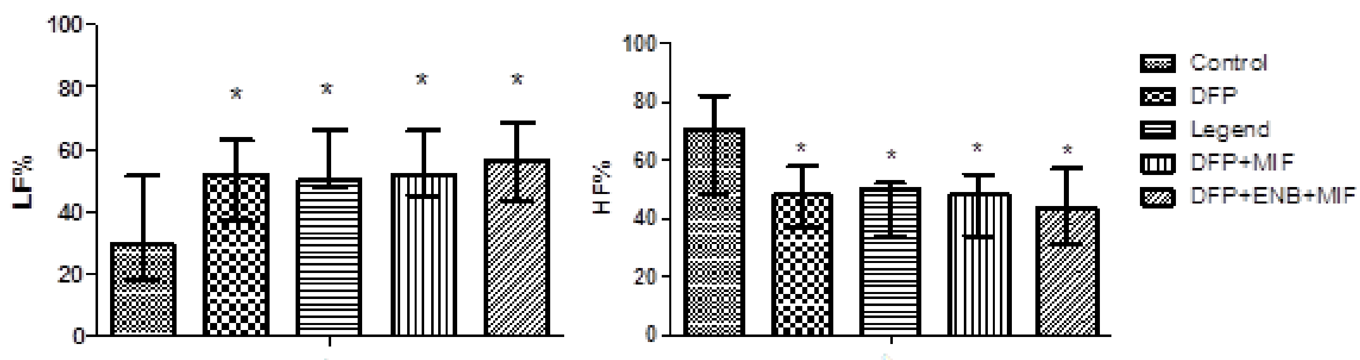


Figure 15- Autonomic function – high frequency (HF), low frequency (LF) balance. Data are expressed in median, 25th percentile and 75th percentile. We performed a Dunn-Bonferroni post hoc method following a significant Kruskal-Wallis (n=10). *=p≤0.05 vs Control. ENB = ENBREL and MIF = MIFEPRISTINE. Drug treatments did not alter the DFP GWI model.